# DISACCHARIDE INTOLERANCE

# AND

# PROTEIN-CALORIE MALNUTRITION

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IN MEMORY OF MY PARENTS

DAVID AND HELEN BOWIE

AND TO

ELAINE

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## CHAPTER I.

## THE INTERRELATIONSHIP OF DIARRHOEA AND MALNUTRITION.


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## CHAPTER I.

### THE INTERRELATIONSHIP OF DIARRHOEA AND MALNUTRITION.

"On a worldwide basis it is estimated that disease and disorders in which diarrhoea is an outstanding manifestation account for 5,000,000 deaths of infants and children every year. These diseases have received remarkably little study in relation to their enormous importance, and views regarding aetiology vary from the conviction that all diarrhoea is due to acute enteric infection to the belief that these conditions are predominantly caused by or related to preceeding nutritional disease".[1]

In the industrially developed countries where malnutrition is uncommon there has been a rapid and consistent decline in mortality from diarrhoea.[2,3] During the period from 1900 to 1951 the number of deaths reported to be due to diarrhoea in the United Kingdom fell from 30,000 to 891.[4] In Scotland in 1915 the mortality from diarrhoea was 57 per 1,000 live births and by 1946 had fallen to 8.5 per 1,000 births.[3] In Cape Town the mortality rate for white children is comparable to the above figures. For the 5 years ended 30 June 1920 it was 28.8 and the year 1959 was only 0.27 per 1,000 live births.[5]

The incidence of the disease in these countries does not show the same decline as the mortality.[6,7] Diarrhoea remains a common cause of illness in infancy but death rarely occurs as a result. It has been stressed that diarrhoeal disease in a previously well nourished individual tends to be an acute but self-limiting disease lasting only a few days.[8]

In most of the industrially underdeveloped countries infantile diarrhoea still presents a major paediatric and public health problem.[9] Gastro-enteritis remains the main cause of death in infants and children.[8] Reliable statistics of mortality rates are not available for many of these countries but in 1952 300 deaths per 100,000 of population in Mexico were due to diarrhoea.[8] In 1955 Jelliffe listed

gastro-enteritis as the major cause of death in infants in the tropics.[10]

In South Africa in general and in Cape Town in particular the mortality in non-white children shows a striking contrast to those of white children during the same period. For the 5 years ended in June 1920 the mortality from diarrhoeal disease in non-white children was 58.7 per 1,000 live births and in 1959 was still 28.1 per 1,000 live births.[3] Thus the mortality from diarrhoeal disease among non-white infants in 1959 was 100 times greater than that in white children.

The striking association between infantile diarrhoea and malnutrition and the association of a high mortality from diarrhoea and malnutrition has been commented on by several workers.[11-13] In a survey of over 1,000 children presenting with dehydrating diarrhoea for parenteral fluid therapy in an out-patient resuscitation room it was found that practically all the children were malnourished and those children who died showed the grossest degree of malnutrition.[14] Other investigators have found similar findings and the mortality rate increases progressively as the weight declines below the expected weight for age.[15-18]

Malnutrition and a high mortality from diarrhoeal disease are undoubtedly associated.[11-18] However, a larger number of severe cases and deaths from gastro-enteritis occur during the summer months and this would suggest that enteric infection is important as the precipitating agent of the disease.[14-19] It has been argued that recurrent or chronic intestinal infection or infestation may be the cause of malnutrition.[20] The problem of whether the malnutrition predisposes to diarrhoeal disease or recurrent diarrhoeal disease to malnutrition is a difficult one. The population groups that show the highest incidence of malnutrition are those which have the lowest standards of hygiene.[21]

Truswell et al[13] have pointed out that if malnutrition is a predisposing cause of severe gastro-enteritis it ought to be possible to find signs which indicate that the child was malnourished before the diarrhoea started. In his series

of patients one half had hypoalbuminaemia. Many of these had short histories so that the bout of diarrhoea could not account for this evidence of malnutrition. Robertson et al[11] showed that of 242 children who subsequently died of gastro-enteriti 83% were below average weight for age before the onset of the diarrhoea. Heights of children with diarrhoea in a survey in Johannesburg were below expected standards in 94%.[12] Privileged members of the same population group were the same height as the standard (Boston anthropometric Chart)[22] and as height is not affected by acute illness and dehydration it would suggest that the children with diarrhoea had long standing pre-existing malnutrition.

Wittmann and Hansen[23] in a study of children presenting with dehydrating diarrhoea did not find a greater frequency of infection in the more malnourished infants of their series. Severe recurrent diarrhoea was nevertheless strikingly more common in the low weight children. They concluded that infection was so frequently present that it must be considered the most common precipitating cause of the diarrhoea but that infection was equally common in well nourished and malnourished children. The malnourished child had recurrent and more severe episodes of diarrhoea. They felt that severe gastro-enteritis is often the result of an abnormal response of a malnourished host to infection or infestation.

In protein-calorie malnutrition diarrhoea is described as an almost constant association of the clinical picture of kwashiorkor.[24-26] Dean[26] has also pointed out that a history of diarrhoea has been an important feature of many descriptions of diseases that would now be called kwashiorkor. Workers in several areas have done bacteriological investigation of the diarrhoea and a high percentage of the children in these series had no recognised intestinal pathogen isolated from the stool.[27-29] Gastro-intestinal infection would therefore not appear to be a satisfactory explanation of the diarrhoea. More recently duodenal intubation of children with kwashiorkor has revealed a high incidence of Giardia lamblia

infestation in Cape Town.[30] Gastro-intestinal infestation may thus play a larger role in the causation of the diarrhoea than was previously thought. Smythe[31] postulated that disturbance or overgrowth of intestinal bacterial flora may play a part in the aetiology of the diarrhoea of kwashiorkor. He found a rapid response of diarrhoea to large doses of broad spectrum antibiotics and nystatin. However, objective data on stool weights and a control series were not included in this study. Almost complete sterilisation of the gut by his therapy may have had its effect by preventing fermentation of carbohydrate of the diet rather than by eliminating infection per se.

Hansen et al[27] have produced objective data documenting the diarrhoea of kwashiorkor. In a study of 85 children with kwashiorkor they showed that the mean stool weight on admission was 4 to 5 times the upper limit of normality for this age. Those patients from whose stools pathogens were cultured had on the average the highest stool weights. Whether the stools contained identifiable pathogens or not, the mean stool weight of kwashiorkor infants only gradually fell to more normal levels over a period of 3 to 4 weeks. Even at this stage the mean stool weights were above the upper limit of normality (100gms/24 hours). They correlated the improvement of the diarrhoea with the rise in serum albumin concentration. It was concluded that there was an association between low serum albumin concentration and high stool weight.

In a child who has died of kwashiorkor striking pathological changes occur in the organs of digestion.[26] The walls of the small intestine are thin and there is gross mucosal atrophy.[32-35] The liver is characteristically fatty.[24,25] Fatty change is most marked in the periphery of the liver lobules but the degree of fatty change may be so intense that little normal tissue may be seen.[26] The pancreas is atrophic and it may be so reduced in size that it resembles a small cord stretching from duodenum to spleen. Acinae may have largely disappeared and the acinar cells left

may be shrunken and contain no zymogen granules. Deficiency of pancreatic enzymes has been described by several workers and on recovery pancreatic function may return to normal very rapidly.[26,29,32,33,36-41] Recent work by Barbezat[30] has raised the possibility that in long continued malnutrition permanent damage to pancreatic function may occur.

The advent of peroral intestinal biopsy has allowed the study of the small bowel mucosa in man and this has lately been done in patients with kwashiorkor. Stanfield et al[42] and Burman[43] have reported the appearance of the small bowel mucosa of kwashiorkor under the dissecting microscope and by histological examination. Although the system used to grade the appearance of the mucosal changes differed in the two investigations their results were very similar. They showed fairly gross changes in the mucosa of the upper small intestine mainly involving villous fusion giving specimens with ridge or leaf shaped villi. There was villous atrophy and broadening with increased cellularity of the substantia propria. Stanfield et al[42] followed some of their subjects for as long as a year after cure and apparent return to normal nutrition. Even at the end of this period abnormal mucosal appearances were present in what were then apparently healthy individuals.

Experimental kwashiorkor produced in the Rhesus monkey has demonstrated very similar changes in the gastro-intestinal tract.[44] The villi of the duodenum and jejunum become shorter and broader and there was a decrease in the crypt/villous ratio. An excess cellular exudate in the substantia propria was not observed and the cellular elements of the villi and crypts showed no morphological abnormality. These authors pointed out that the lining epithelium of the small bowel has the highest cell turnover among the various tissues of the body. These cells would thus be expected to be particularly vulnerable to protein deficiency. They studied the cell kinetics of the small bowel mucosa in protein deficient monkeys using tritiated thymidine.[45] From their studies they concluded that the movement of cells up the villi was slower in protein deficient animals and that this was the cause of the reduction in height of the

villi.

The changes found in the gastro-intestinal tract of individuals with kwashiorkor suggest that disturbance of digestion and absorption must play a part in the causation of the diarrhoea. It has been shown that apparent nitrogen absorption in kwashiorkor is impaired.[46] Fat absorption is also below normal and the absorption index is usually about 80%.[47]

Dean[48] first suggested that the diarrhoea of kwashiorkor appeared when a certain level of carbohydrate tolerance was exceeded. In one experiment intended to demonstrate the value of protein in the arrest of the diarrhoea it was realised that the good results obtained did not depend on the increase of dietary protein but on the decrease of dietary carbohydrate. When the tolerance of carbohydrate was exceeded a violent diarrhoea was produced with highly acid liquid stools. He described the loose stools characteristic of a milk diet in kwashiorkor as small in amount containing flecks and small lumps of yellow material. They smelt sour and were acid in reaction. He later stated[26] that the most spectacular diarrhoeas in kwashiorkor were produced by sugars and stopped by the removal of sugar. He further suggested that this may be due to failure of the requisite enzyme system in the intestine to digest and absorb sugars or that the failure of secretions into the gut may affect the digestion of sugars chiefly by allowing abnormal bacterial growth.[26]

Dietary carbohydrate has previously been incriminated as a cause of diarrhoea. In the early part of the century Finkelstein[49] and others[50,51] developed the concept of fermentative diarrhoea. They considered that the addition of sugar to milk feeds was an important factor in the causation of diarrhoea. Finkelstein[49] developed a "protein milk" for the treatment of sickly infants particularly those with diarrhoea. He also realised that the beneficial results that he obtained with his formula did not depend so much on the increase of protein as on the decrease of carbohydrate in the diet. It is of interest to note that

the infantile mortality rate from diarrhoeal disease when Finkelstein did this work in Europe was very similar to that which pertains in the underdeveloped countries at present. Social conditions and nutritional status in Europe then must have quite closely resembled conditions prevailing among underpriviledged populations in Cape Town today. It is tempting to speculate that the diarrhoeal disease of that time in Europe had a similar aetiology to the diarrhoea occurring in the underdeveloped areas at present.

The relationship of dietary carbohydrate to diarrhoea has recently been clarified. Several factors are responsible for this. The physiology and biochemistry of carbohydrate absorption and digestion have become better understood.[52,53,54] This particularly applies to the absorption of the disaccharide sugars. The locus, distribution and development of the disaccharidase enzymes which split disaccharides into their constituent monosaccharides have now been more clearly defined. Of importance was the description by Holzel, Schwarz and Sutcliffe[55] in 1959 of congenital lactose intolerance in two siblings. These children had severe diarrhoea but became asymptomatic following the institution of a lactose free (i.e. milk free) diet.

Since then numerous reports have appeared in the literature describing intolerance to disaccharides both congenital and acquired, both in children and adults.[56] Of greatest interest in the present context has been the description of secondary disaccharide malabsorption. Disorders and diseases affecting the intestinal mucosa are frequently followed by the reduction of the intestinal disaccharidase activity. This has been demonstrated in a wide variety of diseases including infectious diarrhoea,[57] coeliac disease[58] and giardiasis.[59] Interest in "fermentative diarrhoea" has been revived.

The description of the stools characteristic of a fermentative diarrhoea[60] bears a striking resemblance to the stools of a child with kwashiorkor on a milk diet which from personal observation are liquid and foamy containing flecks of faecal material. They have a sour rather than foul smell. The pathological changes in the

small bowel[32-35] and the biopsy studies[42,43] suggest that malfunction of digestion and absorption are a factor in producing the diarrhoea. The discovery of disaccharide malabsorption as a secondary phenomenon to disorders or disease affecting the intestinal mucosa is of significance.[56,57,59] Dean[26] has presented evidence to suggest that the sugar of the diet played a role in the causation of the diarrhoea. Infection alone does not seem a satisfactory explanation of the long continued and recurrent diarrhoea seen in cases of severe protein-calorie malnutrition. These considerations lead to the postulate that whatever the initial cause of the diarrhoea might be, the chronic diarrhoea of kwashiorkor might possibly be due to an acquired disaccharide intolerance.

## CHAPTER II.

## THE DISACCHARIDES, DISACCHARIDASES AND CLINICAL SYNDROMES BASED ON DISACCHARIDASE DEFICIT.


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## CHAPTER II.

## THE DISACCHARIDES, DISACCHARIDASES AND CLINICAL SYNDROMES BASED ON DISACCHARIDASE DEFICIT.

The physiology and chemistry of disaccharide digestion and absorption have been the subject of intensive investigation over the last few years. Clinical syndromes based on defects in normal digestion and absorption have been described and as a result a more complete understanding of the processes of carbohydrate absorption has evolved. In the investigation and evaluation of the possibility of disaccharide malabsorption occurring in protein-calorie malnutrition it is necessary to review these findings and concepts.

### The Disaccharides.

The disaccharides consist of two molecules of monosaccharides condensed together with the elimination of water.[61] There are four disaccharides of importance in human nutrition:- lactose, sucrose, maltose and isomaltose.[56] Lactose is apparently solely of mammalian origin and occurs in the milk of most mammals.[62] Sucrose occurs naturally in certain plants and is the carbohydrate commonly used in the kitchen.[62] Maltose and isomaltose do not occur to any great extent as such in nature. Starch (amylose and amylopectin) is hydrolysed in the intestinal lumen by salivary and pancreatic amylase releasing maltose and isomaltose.[63-66] Human small bowel mucosa in addition is known to have enzymes capable of splitting palatinose, trehalose and cellobiose.[56] Although of no importance in human nutrition these disaccharides are of interest in studying the biochemistry of disaccharide digestion.[56]

TABLE I.[56]

| | Constituent Monosaccharides | Glycoside Linkage |
|---|---|---|
| **α Disaccharide** | | |
| Maltose | Glucose + Glucose | α 1 - 4 |
| Isomaltose | Glucose + Glucose | α 1 - 6 |
| Sucrose | Glucose + Fructose | α 1 - β2 |
| Palatinose | Glucose + Fructose | α 1 - 6 |
| Trehalose | Glucose + Glucose | α 1 - α1 |
| **β Disaccharide** | | |
| Lactose | Galactose + Glucose | β1 - 4 |
| Cellobiose | Glucose + Glucose | β1 - 4 |

**The Disaccharidases.**

Much of the current knowledge of the specificity and multiplicity of the intestinal disaccharidases in both experimental animals and man has come from the studies of Dahlqvist,[66] Semenza[67] and Auricchio.[68] In the small intestine of man 6 separate α disaccharidases and 2 β disaccharidases have been described.[67,68]

TABLE II.[67,68]

The Disaccharidases and the percentage amount of the specific Disaccharides split.

| Disaccharidases | Percentage of splitting of | | | |
|---|---|---|---|---|
| | Maltose | Sucrose | Isomaltose | Lactose |
| ∝ Disaccharidases | | | | |
| Maltase 1 | 15 | - | 1 | - |
| Maltase 2 | | - | - | - |
| Maltase 3 = Sucrase 1 | 5 | 10 | - | - |
| Maltase 4 = Sucrase 2 | 80 | 90 | - | - |
| Maltase 5 = Isomaltase | | - | 99 | - |
| = Palatinase | | | - | - |
| Trehalase | - | - | - | - |
| β Disaccharidases | | | | |
| Lactase 1 = Cellobiase 1 | - | - | - | 90 |
| Lactase 2 = Cellobiase 2 | - | - | - | 10 |

∝ Disaccharidases; maltase 1 and 2 split only maltose and contribute a relatively small percentage to the total maltase activity in the duodenum and proximal jejunum. There is some evidence that they may contribute more to the total maltase activity in the distal small intestine.[69] Maltase 3 and 4 split maltose and sucrose and are synonymous with sucrase (invertase) 1 and 2. Maltase 3 only contributes a small amount to the total maltase and sucrase activity. Maltase 5 is synonymous with Isomaltase and Palatinase as it splits all these disaccharides. Maltase 4 and 5 constitute the major part of the total maltase activity contributing about 80%.[67,68] Maltase 4 and 5 may be individual molecules but it has been

postulated that they may be one molecule with two active groups. This possibility has arisen because of the simultaneous occurence of sucrase and isomaltase deficiency.[70,75] This either represents a double enzyme deficiency occurring in one individual or it could be explained by the above. A further possibility is that the two enzymes are separate but under the control of one operator gene or "operon".[76] Trehalase is a completely separate enzyme and acts only on trehalose.[77]

Of the $\beta$ disaccharidases lactase 1 is by far the most important constituting about 90% of lactase activity.

<u>The site and distribution of disaccharide absorption and disaccharidases in the intestine.</u>

The classic concept of disaccharide digestion given in most biochemistry and physiology text books is that the disaccharidases are secreted into the intestinal lumen as part of the "mucous entericus".[62] There they split the disaccharides into their constituent monosaccharides which are absorbed.

Borgstrom et al[78] showed that the disaccharidase activity of the intestinal contents is too low to account for the rapid absorption of disaccharides. They suggested that the disaccharidase enzymes might be localised in the mucosal cells. Evidence has accumulated to show that this is so.[66,79,80] Miller and Crane[79,80] have been mainly responsible for the development of the concept of intracellular hydrolysis. By means of in vitro experiments with hamster intestine they demonstrated that hydrolysis occurred in the tissue and not in the medium.[79]

These same authors were able to isolate the "brush border" fraction of the intestinal mucosal cells and showed that virtually all the invertase and maltase activity was in this fraction.[80] Lactase is more soluble than the other disaccharidase enzymes and this presents technical problems in its localisation in the cell.[53] Recently it has also been located in the brush border.[81]

The monosaccharides resulting from the hydrolysis of disaccharides are

absorbed either by an active transport process (glucose and galactose) or by passive diffusion (fructose).[53,54] The active transport mechanism of glucose and galactose although imperfectly understood is known to be Sodium ($Na^+$) dependent.[54] It seems likely that the sodium pump and the sugar pump are parts of the same mechanism.[82] Semenza et al[83,84] have recently shown that the activity of human disaccharidases is also Sodium dependent and suggest that the hydrolysis of the disaccharides and the active transport of the monosaccharides are closely related. Disaccharide hydrolysis is probably located in the outer part of the brush border and the active transport of monosaccharides on the inner part.[54]

From their original studies of digestion and absorption of disaccharides in man Dahlqvist and Borgstrom[66] thought that although lactose and maltose were absorbed in the upper and mid-intestine, sucrose was absorbed mainly in the distal jejunum and ileum. Consistent with this concept were Dahlqvist's[85] findings on the location of the disaccharidases in the digestive tract of the pig. Subsequent work and studies have suggested that this original concept is incorrect. Dahlqvist and Thomson[86] and Blair and Tuba[87] have demonstrated that in the rat invertase activity occurs mainly in the upper small intestine.

In man the distribution of the disaccharidase activities along the intestinal tract has been studied in the newborn.[88] The activity is uniformly high over the whole length of the small intestine except in the duodenum and terminal ileum. In these areas slightly lower levels of activity are found. No significant activity is found in the stomach or large bowel. There is no difference in the distribution of the $\alpha$ and $\beta$ disaccharidases. Trehalase is the single exception where the values of activity remain high in the terminal ileum. No similar study has yet been performed in older children and adults but biopsy studies suggest that a similar distribution will be found.[69]

The development of disaccharidase activity.

The activity of small intestine disaccharidases differs in the foetus and newborn from the adult. In calves, rabbits, pigs and rats, lactase activity develops during foetal life and after birth drops to the lower level of the adult.[89-93] In contrast the ∝ disaccharidase activities are absent or very low at birth and only later rise to adult levels.[93-97] Fomina[98] showed that in man lactase and sucrase are both fully active at birth. Auricchio et al[88] in a study on human embryos, foetuses, premature infants and full term newborns showed that all the glycosidases (disaccharidases) were present by the third month of intra-uterine life. ∝ disaccharidase activities reached maximal values by the 6th to 8th foetal month. The β glycosidases showed a slightly different pattern and only reached maximal values at the end of normal gestation. ∝ disaccharidase activity is present at maximal values at birth and is independent of time elapsed since birth or previous food intake. β disaccharidase activity is at maximal values at the time of normal birth, or if the infant is prematurely born rapidly achieves maximal values and this is independent of milk intake.

After infancy the level of lactase activity usually drops slightly and only rarely are the levels of the first year of life maintained.[67] In most instances the level of lactase activity is sufficient for the normal utilisation of lactose in the diet. The large numbers of reports[69,99-110] documenting a significant percentage of adults who have malabsorption of lactose perhaps signifies that this does not hold for all apparently normal adults.

The Clinical Syndromes Based on Disaccharidase Deficit.

Congenital Malabsorption of Lactose. This condition was first described by Holsel, Schwarz and Sutcliffe[55] and since their original description several cases have been recorded in the literature.[111-115] The disorder is characterised by a fermentative

diarrhoea and secondary failure to thrive commencing shortly after birth. The diarrhoea is more severe in breast fed infants because of the higher lactose content of breast milk. Intestinal disaccharidase activity in the infants studied[114-115] showed an absence or marked diminution of lactase activity whereas the ∝ disaccharidase activities were within the normal range. It is probably an hereditary condition and there are two reports of affected siblings.[52,112] The type of inheritance is as yet unknown. The father of one patient had malabsorption of lactose[112] but as such a condition is not infrequent in the adult he may have had a coincidental acquired intolerance.

Lactosuria is not a feature of the condition. Congenital lactose intolerance associated with lactosuria is a distinct and probably more complex syndrome. It was first described by Durand in 1958[116] and is characterised by vomiting and diarrhoea with the urinary excretion of large amounts of lactose and lesser amounts of other disaccharides and monosaccharides. There is often aminoaciduria and renal acidosis. Several patients have since been described with this disorder.[113,117-122] Many have died despite the institution of a lactose free diet so that it would appear to be a more serious and widespread lesion than that described by Holzel.[55] No intestinal enzyme studies have been carried out in these cases so that it is difficult to speculate on the relationship of the two entities.

Congenital Malabsorption of Sucrose and Isomaltose.

Weijers et al[123,124] first described sucrose malabsorption in 1960 diagnosing the condition by means of oral tolerance tests. With the advent of direct enzyme assay it has been shown that most if not all have an accompanying malabsorption of isomaltose.[70,71] A large number of patients have now been recorded[72-76,106,112, 125-131] and in all who have been extensively studied this association has been shown. Some had no clinical evidence of isomaltose malabsorption and could tolerate starch.[75]

The congenital sucrase/isomaltase deficit is present from birth and a

fermentative diarrhoea occurs as soon as the diet includes sucrose, dextrins or starch.[56] Secondary failure to thrive occurs and associated steatorrhoea and malabsorption of xylose has been reported. The steatorrhoea ceased with the elimination of sucrose from the diet.[73,75] In most patients the histology of the intestinal mucosa has been normal[75,106] and in one exception[76] the histology returned to normal when sucrose was removed from the diet. A discrete disaccharidiuria occurs which is more pronounced during loading tests but it is usually within the physiological range.[70]

The condition appears to be hereditary. There are several records of affected siblings and two of consanguinous parents.[56] Tolerance to sucrose and starch seems to improve with age and this has made study of inheritance difficult.[70] Workers in Australia have recently produced convincing evidence to show that the disorder is inherited in a recessive manner.[132]

No other congenital disaccharide malabsorption syndromes have been described. Congenital malabsorption of maltose as an isolated entity would not be expected to occur. A similar clinical picture to congenital disaccharide malabsorption occurs in malabsorption of the actively absorbed monosaccharides. This is a rare familial condition and as yet has been described from only a few centres.[133-137]

Acquired Malabsorption of Disaccharides.

There would appear to be two distinct syndromes comprising the acquired malabsorption of disaccharides. Acquired lactose malabsorption which occurs in patients in the apparent absence of other disease and symptomatic malabsorption of disaccharides which occurs secondarily to other primary disease of the intestinal mucosa. This separation of the acquired syndromes into two groups may be artificial and will be discussed later.

Acquired Lactose Malabsorption.

Since the description of congenital lactose malabsorption the study of

disaccharidase activities in adults has brought to light a large number of patients both symptomatic and asymptomatic with low levels of lactase activity.[69,99-110] The incidence reported varies from 16 [109] to 55% [107] of the patients studied. Acquired lactose malabsorption is relatively common and is the cause of many if not all the cases of milk intolerance in the adult.[105]

The symptomatology of acquired lactose malabsorption is apparently very variable. Some patients present with signs and symptoms very similar to congenital lactose malabsorption of infancy. The ingestion of small amounts of lactose or milk results in abdominal discomfort and distension and a fermentative diarrhoea. In other there were no symptoms until a diet containing a large amount of milk (e.g. an ulcer diet) provided a sufficient lactose load. Some could tolerate a fairly large lactose load if spread over the day but diarrhoea was precipitated by a comparable load given in a single dose as a lactose tolerance test.[105] In this regard it has been claimed in the past that a significant number of adults will have "flat" blood sugar curves following lactose loading. Many factors may make a sugar tolerance curve flat.[53,138] Now that it is becoming clear that lactase deficit is common it cannot be accepted that normal persons have a "flat" curve.[110] Quantitative mucosal assays must be done to show that lactase deficiency does not exist. Many apparently normal individuals have a lactase deficiency and are not aware of it as they do not ingest lactose in threshold amounts.[105,107,109,110]

Haemmerli et al[105] postulate that the lactose malabsorption of adults is acquired and not the persistence of the congenital lactase deficiency of infants for the following reasons:-

(1) In the patients reported the history of the condition does not date from infancy.

(2) Lactase activity cannot be low in asymptomatic infants. There is a physiological overload of lactose in breast fed infants which results in acid soft stools in normal babies.[88] Any deficiency of lactase activity should manifest at this time. The symptoms of lactose malabsorption are the result of a balance between lactose load

and intestinal lactase activity. Milk intake progressively decreases from infancy to adulthood especially in relation to the amount of absorbing bowel surface available. The lactose load thus progressively diminishes and malabsorption first observed in later life must indicate that lactase activity has decreased to an even greater extent. (3) Children with congenital lactose malabsorption have less pronounced symptoms with increasing age.[105] The mechanism of adaptation to increasing amounts of lactose is as yet unknown. It has been suggested that there is decreased motility of the gut in response to the lactose and products of fermentation.[105] This improvement of the symptoms of the congenital form would suggest that adult lactose malabsorption is acquired later in life.

The pathogenesis of acquired lactose deficiency is unknown. It may be a condition arising later in life without hereditary predisposition or it may be the late manifestation of a genetically predetermined condition. Large scale family and possibly racial studies will help to distinguish between these two possibilities. In all mammals who have been examined intestinal lactase activity declines with advancing age.[89-93] This does not appear to be generally true of man as most adults examined have adequate levels of lactase activity.[69,99-110]

If the condition were genetically determined it might arise in one of several ways. It might be a late clinical manifestation occurring in families who exhibit congenital lactose malabsorption in some members in infancy. The literature contains many examples of congenital lactose intolerance but at present the symptomatic adults reported in these families date their symptoms from childhood.[105] Another possibility is that the adult with lactose malabsorption may simply represent the lowest levels in the normal wide scatter of intestinal lactase activity.[105] A wide scatter has been reported by several authors but this range of "normal" lactase activity includes many who probably are lactase deficient but asymptomatic. McMichael et al[108] have shown that the frequency distribution of lactase activity

demonstrates two populations - one with "hypolactasia" and one with normal lactase activities. More recent work by these authors[139] supports the view that low lactase activities do not represent the lowest tail of the normal lactase range.

It has been suggested that primary lactase deficiency in the adult may be inherited more commonly in certain racial groups. Cuatrecasas et al[107] demonstrated a high incidence in American negroes while Jeejeebhoy et al[140] showed a similarly high incidence in Indians. Cook and Kajubi[141] in a study of the tribal incidence of lactase deficiency in Uganda showed that it was much commoner in certain tribes than others. McMichael et al[139] in a limited number of cases showed a very high incidence of lactase deficiency in Greek Cypriots. Racial differences in the inheritance of lactase deficiency appearing only later in childhood or in adults would explain the variation in the reported incidence by various authors.[109]

If the condition arises "de novo" in later life without genetic predisposition several possibilities have to be considered. Malabsorption of of disaccharides has been shown to occur following on other conditions affecting the small bowel.[57,58,59,112,114,142-147] These may present as malabsorption of lactose and not of all disaccharides.[56] The acquired lactose malabsorption in the adult in the apparent absence of other disease may be the persistence of secondary malabsorption as a sequel of transitory small bowel disease. The division of acquired disaccharide malabsorption into two groups may therefore be fallacious and both may be manifestations of secondary malabsorption due to small intestinal damage of other cause. It is difficult to understand why a generalised disorder should cause damage to only one enzyme but lactase activity is normally lower than the $\alpha$ glycosidases and a decrease in all enzymes would first become manifest by impaired lactose tolerance. The apparent permanence of the defect is also difficult to explain as a result of non-specific injury. However, lactase does appear to be susceptible to permanent damage. Serial biopsy studies in patients with sprue[148] and children with coeliac disease[144] show that

$\alpha$ glycosidase activity returns to normal levels in most cases with clinical recovery. Lactase levels remain relectively low in a large percentage of such cases.[144,148]

Enzyme adaptation is a further hypothesis that has been suggested to explain lactose malabsorption arising "de novo" in the adult. This supposes that the individual stops drinking milk for no obvious reason and as consequence loses his lactase activity due to lack of substrate supply. Adaptation of pancreatic enzymes has been shown to occur in rats[149] but similar studies have not shown this to be true of lactase.[89-91,150-152] Only one group of investigators[153] have reported increases in specific intestinal lactase activity in adult rats fed on a lactose diet, Circumstantial evidence against adaptation occurring in respect of the disaccharides is provided by the enzyme trehalase in man.[77,115] Trehalose is a sugar occurring only in young mushrooms.[154] The intake of trehalose in man must be exceedingly small, yet specific trehalase activity persists throughout life.

A final suggestion has been that acquired enzyme inhibitors may be the explanation. This would have to be a specific lactase inhibitor and no evidence for its existence has been found.[99]

A satisfactory explanation of the aetiology of acquired lactose malabsorption remains to be found and the evidence available only allows for the above speculation. More than one of the above hypotheses may eventually be found to operate. Certain individuals or racial groups may have a genetically inherited tendency to low lactase activity levels in the adult. Such an individual subjected to non-specific small intestinal mucosal damage may then either lose lactase activity entirely or have it reduced to such a low level that symptoms appear. Study of secondary disaccharide malabsorption may yield clues to the correct explanation.

<u>Secondary Disaccharide Malabsorption</u>.

Disorders and diseases affecting the intestinal mucosa are frequently or perhaps always followed by a reduction of the intestinal disaccharidase activity.

Secondary disaccharide malabsorption has been demonstrated associated with infectious diarrhoea,[57,142,143] in sprue[148] and coeliac disease,[144] cystic fibrosis,[114,145] ulcerative colitis,[155] regional enteritis,[155] Giardia lamblia infestation,[146] irritable colon syndrome,[156] protein-calorie malnutrition,[157,158] post-gastrectomy,[159] intestinal resections,[103] blind loop syndrome and abetalipoproteinaemia.[147]

The malabsorption of disaccharides is of all disaccharides but many present clinically with lactose malabsorption as the dominant disorder.[56] Recovery from secondary disaccharidase deficiencies may be slow and lag behind the recovery from the primary condition.[56,144] Recovery of lactase activity may be much slower than that of the disaccharidases and the deficiency of lactase activity may be permanent.[144,148] The importance of this factor has been discussed in considering the aetiology of primary lactase deficiency in the adult. Secondary disaccharidase deficiencies are more common in practice than the congenital deficiencies. The presence of disaccharide malabsorption may make the treatment of the primary disorder more difficult. Recognition and dietary elimination of disaccharides particularly lactose may allow for more rapid recovery from the causative primary condition responsible for the secondary disaccharide malabsorption.

## SUMMARY.

The association of a high mortality from diarrhoeal disease and malnutrition has been frequently noted. In the industrially developed countries where the nutritional status of the population is satisfactory diarrhoeal disease is still a common cause of illness in infants but the mortality has fallen to very low figures. In contrast, in the underdeveloped countries where malnutrition is common, diarrhoeal disease is a major cause of death and chronic morbidity in young children.

In the severest forms of protein-calorie malnutrition diarrhoea is described as part of the clinical picture. Gastro-intestinal infection with specific pathogens, only occurs in a minority of such cases. Parasitic infestation is more

common than used to be thought and overgrowth of intestinal bacterial flora may be a factor. The greater incidence of diarrhoeal disease in the summer months appears to support an infective factor in the causation of the diarrhoea. Infection and infestation are as common in the better as in the more malnourished section of the population but it is in the malnourished that chronic and recurrent diarrhoea and a high mortality occur. Some factor other than infection must be operative.

The pathological changes found in the gastro-intestinal tract of children who have died of kwashiorkor suggest that disturbance of digestion and absorption occur and these have been demonstrated. Changes in the small intestinal mucosa have also been shown in biopsy studies and have been reproduced in experimentally malnourished animals. Intolerance and malabsorption of sugar in the diet has been suggested as a possible factor in causing diarrhoea in malnourished children.

The physiology and biochemistry of carbohydrate absorption and digestion is becoming more clearly understood. In recent years a great deal of interest and investigation have been centered on the disaccharides. Clinical syndromes based on congenital or acquired defects of enzyme systems involved in the absorption and digestion of disaccharides have been described and extensively studied. Isolated lactose malabsorption occurring in the older child or adult presents a particular problem. There is strong suggestive evidence that it may be genetically determined and show a higher incidence in certain races but the role that intestinal disease plays in its causation has not been clarified. Generalised reduction of disaccharidase activities often presenting clinically as malabsorption of lactose have been described following on other primary diseases of the small intestine.

The fermentative diarrhoea of disaccharide malabsorption bears a striking resemblance to the diarrhoea of protein-calorie malnutrition. This suggests that the latter may be a fermentative diarrhoea and a secondary disaccharide malabsorption following on primary damage to the intestinal tract produced by the protein malnutrition.

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## CHAPTER III.

### DESIGN AND PURPOSE OF THE EXPERIMENT.

1. **To show that children with severe protein-calorie malnutrition have a fermentative diarrhoea.**

The symptoms and diagnosis of fermentative diarrhoea have been described by several authors.[56,60,105] Diarrhoea depends on the ingestion of carbohydrate or of a specific disaccharide. On removal of the offending disaccharide from the diet the diarrhoea will cease.[56,60] There are no specific signs on physical examination. The abdomen may be distended and gas filled coils of gut may be detected. Two mechanisms have been postulated to explain the diarrhoea of disaccharide malabsorption.[105] The large amount of unabsorbed and unabsorbable disaccharide passing through the intestine takes with it water causing an osmotic diarrhoea.[105] The unabsorbed disaccharide undergoes bacterial degradation in the lower reaches of the small intestine and in the colon. The products of fermentation apart from their osmotic effect cause irritation of the gut and increased motility.[105] Supportive evidence in favour of a diagnosis of fermentative diarrhoea can thus be found by the detection of sugars and of the products of fermentation in the stools.[60]

The detection of sugar in the stool may be done by paper chromatography of the stool water. A simpler method has been described by Kerry and Anderson using "Clinitest" tablets (Ames).[160] The evidence of fermentation detectable in stools is that they contain large amounts of low molecular weight organic acids.[124] Of these acids, lactic acid is of particular importance.[124] It can easily be determined by chemical[124] or enzymatic methods;[112] it is present in normal faeces only in small amounts and in fermentative diarrhoea the lactic acid content of the stool is increased many fold.[56,124] Quantitative assay is therefore a valuable, simple and practical

method of demonstrating a fermentative diarrhoea. A simpler but far less reliable method is to measure the pH of the stool. Normal stools have a pH about 7 whereas stools in fermentative diarrhoea usually have a pH of 5.5 or less.[56] Factors other than the excretion of low molecular weight organic acids may influence the pH of the stools giving misleading results.[60]

Accordingly studies were set up to show that children with protein-calorie malnutrition had diarrhoea when given carbohydrate (disaccharide) and that this promptly ceased when the carbohydrate was removed from the diet. Analysis of the stools of these children was undertaken to demonstrate the presence of sugar and lactic acid and to study the pH changes.

2. <u>To show that the fermentative diarrhoea was due to disaccharide malabsorption.</u>

Two methods are available for confirming the diagnosis of disaccharide malabsorption in a fermentative diarrhoea.[56] Oral disaccharide loading tests may be used. These must be performed when the patient is diarrhoea free i.e. in a period when the patient is not receiving the suspected disaccharide.[56,60] After oral loading of the non-absorbable disaccharide there is little or no rise in blood glucose levels and the test is followed by diarrhoea with acid stools containing the disaccharide or the product of bacterial degradation of that disaccharide i.e. its constituent monosaccharides and low molecular weight organic acids particularly lactic acid.[70] When the constituent monosaccharides of the disaccharide are given there is a normal increase in blood glucose levels and the loading test is not followed by an episode of fermentative diarrhoea.[56,70]

The second method of confirming the diagnosis is by direct assay of the disaccharidase enzyme activity of the small bowel mucosa.[69] Mucosa is obtained by per oral biopsy of the duodenum or jejunum and is incubated under controlled conditions with the appropriate substrate.[69,77] The amount of glucose produced from the substrate by the enzyme activity of the mucosa is then measured. Enzyme assay should always be

combined with the appropriate oral tolerance tests as the assay results may occasionally be misleading.[56] The sample of mucosa obtained may not be representative of the entire small bowel as in coeliac disease where the proximal part of the intestine is more effected than the distal.[144] In cases where disaccharide malabsorption occurs because of shortening of the length of gut (as in massive resection) the disaccharidase levels of the remaining mucosa may be normal.

Studies were therefore performed on patients with protein-calorie malnutrition after control of the diarrhoea using oral disaccharide loading tests and comparing the increments of blood sugar to that obtained following loading with the constituent monosaccharides. In some cases these were combined with mucosal biopsy and direct enzyme assay of disaccharidase activity.

3. <u>To investigate whether the malabsorption was of all common dietary disaccharides or only of lactose.</u>

Secondary disaccharide malabsorption may be a generalised malabsorption of all disaccharides[57,142-146] but frequently the malabsorption of lactose dominates the clinical picture.[56]

Oral disaccharide loading tests of all the common dietary disaccharides were performed and the absorption of the various disaccharides compared. In addition mucosal enzyme assay gave a direct comparison of the various disaccharidase activities to normal values reported in the literature.[69,109,161]

4. <u>To investigate the effect of disaccharide (lactose) malabsorption on the absorption of other nutrients.</u>

In some cases of congenital sucrose/isomaltose malabsorption there has been an accompanying steatorrhoea.[73,75] This has returned to normal after elimination of the malabsorbed disaccharides from the diet.[73,75] Children with protein-calorie malnutrition have been shown to have diminished nitrogen absorption[46] and a mild

steatorrhoea.[47]

Balance studies were therefore performed while receiving lactose and when lactose was excluded from the diet to study the effect of control of diarrhoea on the absorption of nitrogen and fat. In addition xylose tests were done to study the effects on simple carbohydrate absorption.

5. <u>To determine whether the malabsorption of lactose was transient or not</u>.

In several of the reported cases of secondary malabsorption of lactose following on infectious diarrhoea the acquired enzyme deficiency has persisted for several months.[57,142,143]

Follow up studies of a limited number of the children investigated were undertaken while keeping them on a good protein intake. Persistence or reversal of lactose malabsorption was studied by means of oral lactose loading tests and repeated small bowel mucosal biopsy and enzyme assay.

6. <u>To attempt to define the relationship of infection and the degree of malnutrition to the causation of lactose malabsorption</u>.

In the community from which the children with severe protein-calorie malnutrition were drawn, infective diarrhoea and Giardiasis are common. Both these conditions have been reported as being the primary aetiology in secondary disaccharide malabsorption.[57,142,143,146] The combination of protein-calorie malnutrition and one or both of these other conditions might account for a high incidence of secondary disaccharide (lactose) malabsorption. Examination of the stools for parasites and bacteriological culture for pathogens was therefore done in all cases. The degree of malnutrition of those with lactose malabsorption was compared to those without to see if this factor had any significance.

## <u>SUMMARY</u>.

Studies were undertaken to show that children with protein-calorie

malnutrition had a fermentative diarrhoea and that this was due to disaccharide malabsorption. The studies were also designed to show whether the disaccharide malabsorption was of all dietary disaccharides or of lactose only and whether the disaccharide malabsorption had any effect on nitrogen and fat absorption. An attempt was also made to see whether the malabsorption of lactose was transient and temporary and if there was any relationship to gastro-intestinal infection or to the degree of malnutrition.

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## CHAPTER IV.

## MATERIAL AND METHODS.

**Clinical Material:**

The investigations to be presented were undertaken on 47 children presenting at the out-patient department of the Red Cross War Memorial Children's Hospital with severe protein-calorie malnutrition.

**Criteria for the selection of patients:**

1. The presence of severe protein-calorie malnutrition. The indices used in making a diagnosis of protein-calorie malnutrition were :-

a) A history of a diet deficient in protein.

b) The weight of the child in relation to his chronological age. All children selected were underweight for age by the standards of the Boston Children's Medical Center percentile chart.[162] All were below the 3rd percentile of weight of this chart.

c) Other signs of malnutrition. The diagnosis of kwashiorkor was made on the presence of oedema in an underweight for age child. In many cases other features such as mental apathy, typical skin lesions and hair changes were also present. In children diagnosed as nutritional marasmus there was no oedema and practically no subcutaneous fat.

d) Low serum albumin. Hypoalbuminaemia below 3.5 gm% was considered essential for the diagnosis of severe protein-calorie malnutrition.

e) The exclusion by examination and special investigation if necessary of the non-dietary causes of failure to thrive and oedema.

2. Only male children were studied because of the greater ease of separate urine and stool collections in metabolic studies in this sex.

Children were not included in this study if (a) they had gross underlying

disease of any sort; (b) they were considered to be too ill to allow investigation; (c) they were much older than the average age of children with protein-calorie malnutrition in this community and difficulties with investigation and particularly prolonged periods on a metabolic bed were anticipated.

Routine investigation and treatment of all patients:

X-ray chest and Mantoux or Heaf testing was done on all children to exclude tuberculosis. All were admitted to a metabolic ward under the clinical care of the investigator. Penicillin and Sulphadiazine were given for the first seven to eight days after admission. No specific therapy was given for any infection or infestation other than the above during the period of investigation. Examination of stools for parasites and bacterial pathogens were carried out in the hospital routine pathological laboratory on admission.

On the day of admission the patients were put on half isotonic Darrows solution with 2½% dextrose. The day following admission this was stopped and the patient put on a diet in accordance with the experimental plan. If dehydration was present on admission or occurred at any time during the period of investigation hydration was maintained by means of intravenous half isotonic Darrows solution with 2½% dextrose. Supplementary potassium chloride was given orally to all children and continued throughout the investigation.

Blood was taken soon after admission for the estimation of serum proteins and for the determination of the haemoglobin value, packed cell volume and the estimation of mean corpuscular haemoglobin concentration.

## EXPERIMENTAL PLAN.

The investigation was undertaken in two series and the diets, dietary manipulations and sequence of detailed investigations differ in the two series.

Series I.

This investigation was carried out from January to December 1963.

Twenty-seven children with severe protein-calorie malnutrition were studied. Twenty-four of these had the characteistic signs of kwashiorkor and three had nutritional marasmus.

In accordance with the feeding schedule on which they were placed they were divided into two groups.

Group I.

This consisted of 16 children, 14 with kwashiorkor and 2 with nutritional marasmus. After the initial period on half-strength Darrows solution they were given milk feeds. This was changed after three days to a carbohydrate-free formula. (vide infra)

Group II.

This group consisted of 11 children, 10 with kwashiorkor and one with nutritional marasmus. After the initial period on half-strength Darrows solution they were fed the carbohydrate-free diet. This was changed after three days to milk feeds.

Stool weights, stool lactic acid and stool sugar content were recorded during these two periods on different diets. D-xylose absorption tests were performed on 6 of the children in Group I (4 with kwashiorkor and 2 with marasmus). Two tests were performed on each child, the first on the day of admission and the second after the diarrhoea had been controlled with the carbohydrate-free diet.

Following the dietary manipulations in 5 of the 6 children on whom D-xylose absorption tests had been performed, oral carbohydrate tolerance tests were done. These children were kept on the carbohydrate-free diet so that they remained diarrhoea free. Glucose/galactose and lactose tolerance tests were performed.

Series II.

This study was carried out from July 1964 to April 1966. Twenty children were studied and all had the characteristic signs of kwashiorkor. All were studied in a similar manner.

Feeding schedule. After the initial period on half-isotonic Darrows solution and 2½% dextrose they were placed on full strength full cream milk feeds. After three days this feed was changed to a disaccharide-free formula (vide infra) for four days. Following this the feed was changed back to full cream milk feeds for a further four days.

Stool weights, stool lactic acid and sugar content were recorded during these three different diet periods. D-xylose absorption tests were performed on all but one child in this series. Three tests were performed on practically all patients, the first during the first period of milk feeding, the second while on the disaccharide-free diet and the third while on the second period of milk feeds. Nitrogen and fat balances were also performed during these three periods of changing diets.

Following the final period of milk feedings the child was put on to the disaccharide-free formula to control diarrhoea. Glucose/galactose, lactose, sucrose and maltose tolerance tests were done in this order with at least a day between each tolerance test. After completion of the tolerance tests biopsy of the small intestinal mucosa was performed on the last 11 of the 20 patients studied. Disaccharidase enzyme assay was performed on the mucosa so obtained.

DIETS.

Carbohydrate-free diet:

This consisted of raw eggs and cream. The feed was made up according to the following formula.[163]

| | | | |
|---|---|---|---|
| Egg - raw beaten up | - | 42 gm. | ± 100 cals. |
| Fresh cream | - | 7 ml. | |

One egg weighs approximately 60 gms. Feeds were prepared so that the child received approximately 100 cals. per kilogram body weight. Volume was made up with the addition of water and saccharine for sweetening. Five grams of special salt (vide infra) was added to each days feed.

Example: An 8 kilogram child was given a carbohydrate-free formula containing 800 calories. This was divided into bottle feeds of 120 ml (4 oz) x 8 = 960 ml. (32 oz) total volume. To give approximately 800 calories.

| | | | |
|---|---|---|---|
| Egg | 42 gm. | x 8 | = 336 gm. |
| Cream | 7 ml. | | = 56 ml. |
| Special salt | | | = 5 gm. |
| Water | | | = 580 ml. |

Disaccharide-free diet.

This consisted of Casein hydrolysate (Casilan), cream and glucose in the following proportions:-

| | | | |
|---|---|---|---|
| Protein (Casilan Glaxo) | - | 40 gm. | (38%) |
| Glucose | - | 30 gm. | (32%) |
| Fat (Thick cream) | - | 52 ml. | (25%) |
| Special salt | - | 5 gm. | ( 5%) |

Diluted with 800 ml water to make a formula. The Casilan, glucose and water were mixed until blended and then the cream added and the mixture again beaten until thoroughly mixed.

Special Salt Formula.

| | | | |
|---|---|---|---|
| Calcium carbonate | - | 50 gm. | (10%) |
| Di-potass.hydrophosphate | - | 165 gm. | (33%) |
| Di-calcium phosphate | - | 35 gm. | (7%) |
| Sodium Chloride | - | 200 gm. | (40%) |
| Mag. sulphate | - | 50 gm. | (10%) |

## CLINICAL AND LABORATORY METHODS.

STOOLS:

During the period when stool collections were made the children were nursed on metabolic beds. These beds allowed for the separate collection of stools and urine.

Stool weight - On the three days prior to and the three days after changing the diet (but not including the day of change) the stools were collected and weighed. The wet stool weight passed per 24 hours was measured. Stools were collected immediately and frozen so that evaporation was avoided. Stool weights are presented as an average daily value for the three days before and after changing diets. Therefore in Series I two sets of stool weights and in Series II three sets of values are available.

Stool lactic acid content: - Stools collected for stool weight were used. These were kept refrigerated until the estimation of lactic acid content was performed. All stool passed in each 24 hour period was thoroughly mixed in an electric blender. An aliquot of this stool was then taken and diluted with water. The lactic acid content of this specimen was then determined by the colorimetric method described by Barker and Summerson.[164]

Protein was precipitated by the addition of Trichloroacetic acid. The protein free supernatant fluid was then treated by a copper hydroxide-calcium hydroxide procedure to remove interfering material (e.g. glucose). The lactic acid was then converted to acetaldehyde by heating in concentrated sulphuric acid and the acetaldehyde determined by its colour reaction with p-hydroxydiphenyl in the presence of cupric ions. The colour was read in a colorimeter with a filter having a peak transmission at 560 mμ. A standard of known lactic acid content was run simultaneously. All estimations were done in duplicate and the results obtained were expressed as grams of lactic acid per 24 hours.

Chromatography of stool for sugars:

This was done on the 24 hour stool samples collected for stool weight and lactic acid content. A simple paper chromatographic technique employing Whatman No.1 paper and ethyl acetate and pyridine as solvent was used.[165] A protein free filtrate of stool was obtained by ultrafiltration through cellulose tubing. Sugar spots were visualised by using a developing reagent containing acetone diphenylamine, aniline and phosphoric acid. Standard solutions of disaccharides (lactose, sucrose and maltose) and monosaccharides (glucose, galactose, and fructose) were run simultaneously to assist identification of any sugars demonstrated in the stool sample.

Stool pH.

This was measured on the samples on which stool lactic acid content was estimated. pH was initially determined using a Beckman direct reading pH meter. Simultaneous determinations using nitrazine pH papers (Merck) showed that this simpler method gave comparable results and most of the pH determinations were subsequently done in this way.

Bacteriology:

Stool samples collected on admission were sent to the routine hospital pathology laboratory for examination for parasites and bacterial culture. These stools were reported on by the hospital pathologist or his assistants.

ABSORPTION STUDIES:

D-xylose Absorption.

D-xylose was given after an 8 hour fast in a dose of 0.5 gm. per kilogram body weight. A total dose of 5 gm. of xylose was not exceeded. In the first series xylose absorption was measured by estimating the amount of xylose excreted in the urine for the first 5 hours following the test dose.[166] In the second series urinary excretion of xylose was measured over the first 5 hours[166] and over 24 hours[167] after administration. Water was given to drink two hours after the test dose to ensure a good

urine flow. All urine passed during the collection periods was frozen immediately. In the second series blood xylose levels were estimated immediately preceeding and 30, 60, 90 and 120 minutes after giving the xylose.[168,169]

The xylose content of the urine was measured by the method of Rice and Roe.[170] This method is based upon the fact that pentoses form furfural when heated in acid solution. The furfural thus formed reacts with p-bromoaniline to form a pink-coloured complex which can be measured photometrically. The urine was diluted to 1 to 200 with distilled water and the 2% p-bromoaniline solution added. This was then heated to 70°C for 10 minutes and after cooling and keeping in the dark for 70 minutes was read at wave length 520 mμ on a Klett-Summerson colorimeter. Unheated blanks and xylose standards were run simultaneously.

Blood xylose was estimated in a very similar manner.[168,169] Capillary blood 0.2 ml. was placed in 1.5 ml. of distilled water. Protein precipitation was carried out by the addition of 0.2 ml. barium hydroxide followed by 0.2 ml. zinc sulphate solution. The supernatant solution was then treated as for the estimation of xylose in the urine with the exception that a 5% p-bromoaniline solution was employed.[169] Xylose standards and reagent blanks were run simultaneously.

NITROGEN BALANCE AND ABSORPTION:

Nitrogen balance and absorption was performed on all children in Series II during the three day periods while they were on milk, disaccharide-free and milk feeds again. The day of changing the diet was excluded from these estimations. The method used has been previously described from this unit with the exception that carmine markers were not used.[171] Stools were collected as described for stool weights and estimation of stool lactic acid content as this was being done simultaneously. Proportional aliquots of the stool specimens for 24 hours were taken and at the end of the three day period these were pooled, thoroughly mixed and analysed. Food intake

was measured by weighing all bottles before and after feeds. Aliquots of each days feed were analysed for nitrogen content. Urine was collected under toluene and 24 hour specimens frozen. An aliquot of the pooled specimen for 3 days was analysed.

Nitrogen content of the aliquots of feeds, stool and urine was estimated by a micro-Kjedahl technique.[171] The digestion mixture was that used in Perrin's macro-method and contained mercuric oxide and potassium sulphate.[172] All analyses were done in duplicate.

Fat balance and absorption:

Fat balance and absorption was performed on all children in Series II at the same time as nitrogen balance and absorption was done. The fat content of the diet and stools were determined by the method of van der Kamer et al.[173] Aliquots were taken from each days diet and the total amount of intake determined as in the nitrogen balances. The stool specimens used were those collected for lactic acid and nitrogen analysis.

Aliquots of the feeds and faeces were saponified with concentrated potassium hydroxide in ethanol. This gave a solution containing the soaps derived from neutral fats, fatty acids and also soaps originally present. The addition of hydrochloric acid liberated the fatty acids and they were then extracted with petrol ether. The fatty acids were then determined by titration against an alkali.

CARBOHYDRATE TOLERANCE TESTS:

In the carbohydrate tolerance tests the children were fasted from 4 to 6 hours before the test. The disaccharides were administered orally as a ± 10% solution in a dose of 2 grams per kilogram body weight. Where the constituent monosaccharides (glucose and galactose) of lactose were given each monosaccharide was administered in a dose of 1 gram per kilogram body weight.

Capillary blood was collected from the heel in an 0.2 ml. pipette in the

fasting state, and at 30, 60, 90 and 120 minutes after giving the sugar. The blood was transferred directly into sodium fluoride solution (0.6 mg. of sodium fluoride per tube). Barium-zinc filtrates were analysed in duplicate by the Somogyi-Nelson technique and "true blood sugar" determined.[174,175] Analysis was usually done on the day of the test. Where this could not be done the specimens were precipitated and frozen, and the analysis done the following day. Glucose standards were measured with each set of determinations.

In the comparison of absorption of a disaccharide and the monosaccharides the technique of absorption ratios has been used.[107] "Absorption" was calculated by measuring the area under each resultant blood sugar curve from fasting to 60 minutes. The "absorption" of the disaccharide was then compared to the "absorption" of glucose and galactose and the result expressed as a percentage of the area under the curve following glucose/galactose administration. All disaccharides have been compared to glucose/galactose as it became obvious early in the investigation that the malabsorption was of lactose only and not of sucrose and maltose. (see Fig.I)

MUCOSAL BIOPSY AND ENZYME ASSAY:

Intestinal Biopsy.

The instrument used for per-oral biopsy was the Paediatric Watson capsule with a 2 mm port. This instrument was used after many attempts to biopsy the small intestine with a Rubin multipurpose biopsy tube. Great difficulty was encountered with the Rubin tube and in only a small number of many attempts was it possible to get the instrument through the pylorus. Attempts to pass this tube under direct vision using an image intensifier were also unsuccessful and it was concluded that the Rubin tube was too inflexible to use on small children.

In using the Watson capsule the following technique was employed. The polythene tube of the biopsy capsule was threaded through a size 14 Rusch radio-opaque

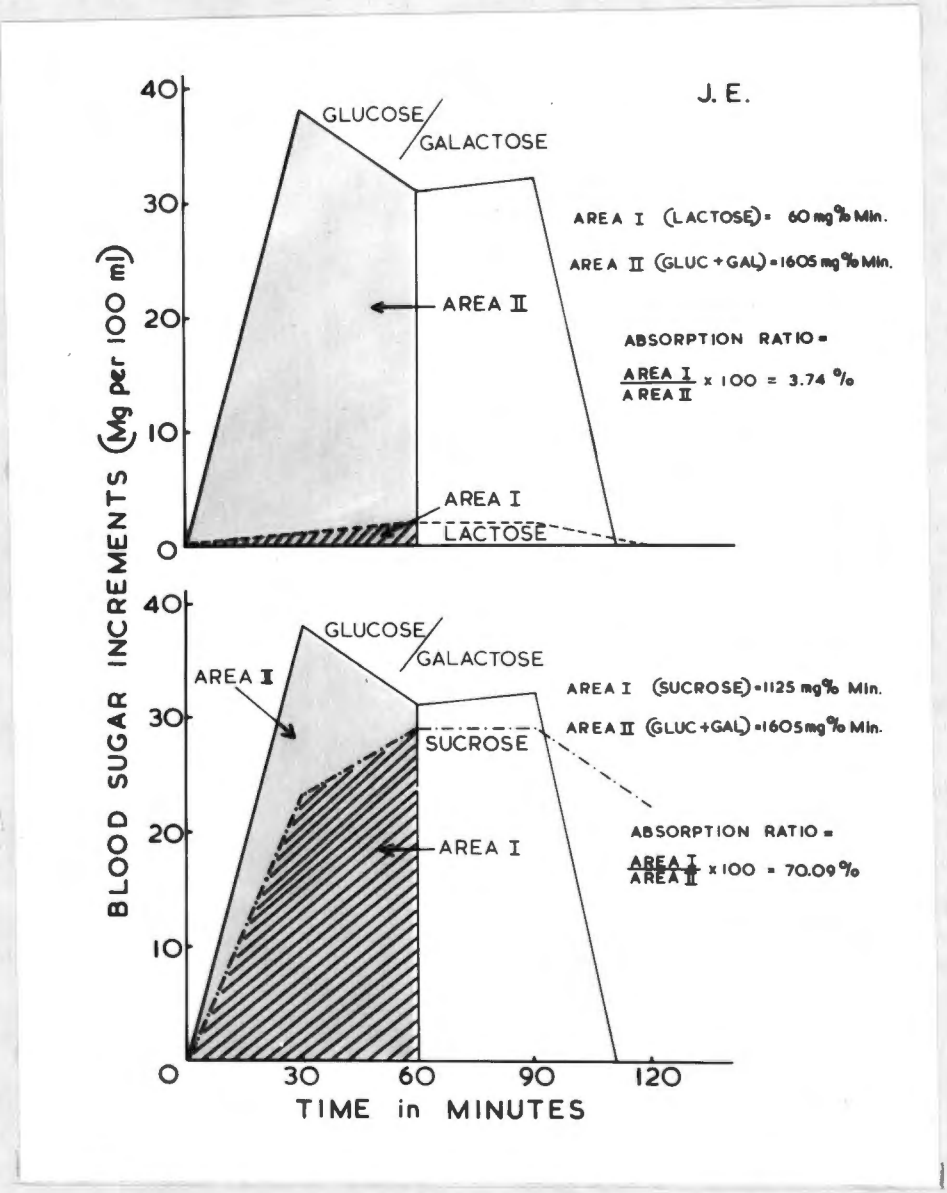


**FIGURE I.**

**Blood sugar increments following administration of glucose/ galactose, lactose and sucrose. Illustration of the method of deriving absorption ratio.**

tube and the capsule passed at the end of this tube. From 6 p.m. on the night prior to the biopsy the patients were given feeds of ½ isotonic Darrows solution and 2½% dextrose. Chloral hydrate (45 mg. per kilogram body weight) was given at 10 p.m. At 11 p.m. the tube was lubricated with a local anaesthetic jelly and introduced via the mouth into the stomach. The child was nursed lying on the right side and the nursing staff instructed to advance the tube approximately 1½ inches every hour during the night. A straight X-ray abdomen taken the following morning checked on the position of the tube and capsule. These were then gently flushed with 3 ml of normal saline followed by a few millilitres of air. The capsule was then fired by attaching a 10 ml. hypodermic syringe to the end of the polythene tube and applying negative pressure. This was repeated several times to ensure that the capsule had fired.

The failure rate of the duodenal intubation using this technique was approximately 4%. Failure of the capsule to fire once it was in the duodenum occurred frequently initially. Lessening the tension applied to the rubber diaphragm when assembling the capsule soon overcame this difficulty and the failure to fire rate also dropped to about 4% of attempts. No complications of the procedure were noted in the 11 patients in this series who were biopsied. As a precaution all children were kept on a half hourly pulse chart for 12 hours after the procedure and full feeds were only resumed the next day.

Enzyme Assay:

The biopsy specimens were taken directly from the biopsy capsule and hermetically sealed in "parafilm". These were then placed in a deep freeze and kept at -10°C until the time of enzyme assay.

After thawing and weighing the mucosa was homogenised by hand in the cold using a glass homogeniser of the Potter-Elvehjem type with 0.9% saline. This

homogenate was used for the determination of lactase, sucrase and total maltase activity after suitable dilution with 0.9% saline. The protein content of the mucosa was also determined from the homogenate.

Assay of disaccharidase activities was performed by the modification of Dahlqvist's[77] method described by Auricchio et al.[69] The mucosal homogenate was incubated at 37°C and a pH of 5.8 with the appropriate substrate solution at 0.028 Molar concentration for 60 minutes. The substrate solutions used were lactose, sucrose and maltose. The liberated glucose was determined with a Tris glucose oxydase reagent. Under these conditions one unit of disaccharidase activity hydrolyses 1 µ mole of substrate per minute.

The protein content of the mucosa was determined by a modification of the Lowry procedure.[176,177]

Disaccharidase activities have been expressed as units (µM substrate split per minute) per gram wet weight of mucosa and per gram mucosal protein content.

Serum Proteins:

These were measured by a biuret method (Wolfson et al)[178] standardised by the Kjeldahl nitrogen procedure. Serum albumin was separated by the ether centrifugation method (Kingsley)[179] after precipitating the globulin with 27% sodium sulphate at 37°C (Milne).[180]

Haematological Methods:

Haemoglobin: - Venous blood 0.02 ml. was pipetted into 5 ml. of ammonia in water and the haemoglobin level read by the oxyhaemoglobin method. A Klett-Summerson colorimeter previously calibrated against standard haemin and cyanmethaemoglobin solutions was used.

Packed cell volume: - heparinised micro capillary tubes were filled with blood and sealed at one end with plasticene. They were then spun for 5 minutes at 11,000 r.p.m. in an International Micro-Capillary centrifuge and read.

# APPENDIX.

## Clinical, laboratory and statistical procedures performed by the author.

1. The selection of patients for the investigation.
2. The clinical management, treatment and follow-up of the children.
3. Blood taking by venipuncture for estimation of serum proteins and haemoglobin values.
4. Supervision of all stool collections and changing of diets.
   Miss C. Freesemann, chief technician in the Unit supervised the weighing, mixing, storage and aliquot taking of the stools.
5. The composition of the Carbohydrate-free and Disaccharide-free diets.
6. Supervision of diet changes with the assistance of Miss Freesemann.
7. All the xylose tests including giving the xylose, taking od capillary blood and supervision of urine collection.
8. All the carbohydrate tolerance tests including giving of the sugars, capillary blood collection and calculation of absorption ratios.
9. Development of intestinal biopsy technique. The majority of the biopsies were performed by the author but Dr. G.O. Barbezat was trained in the technique and performed some.
10. Statistical analysis, computation of means, standard deviations and t-tests.
11. Laboratory methods.
    With the exception of the stool lactic acid determination the following laboratory methods were developed in this unit by the author for the purposes of this investigation. All the initial determinations were done personally by the author. As the number increased technicians attached to the C.S.I.R. Clinical Nutrition Unit were trained in the methods by the author and performed the bulk of the investigations.

The stool lactic acid method had been is use before the start of this investigation. It had not been used for a time and was restarted by the author.

The laboratory methods used in this investigation but not detailed in the following section had all been in routine use in the Unit for some time.

# XYLOSE TEST.

## REAGENTS.

1. **Xylose standard.**

   Stock solution - 1 gm D (+) Xylose in 100 ml saturated benzoic acid.

   Saturated benzoic acid - 4.2 gm per litre or 0.4 gm per 100 ml.

   Working solution - 0.5 ml stock solution in 100 ml distilled water.

   (0.05 mg xylose per 1.0 ml)

   (0.75 mg xylose per 1.5 ml)

2. **Glacial acetic acid saturated with thiourea.**

   Thiourea ± 4 gm.

   Glacial acetic acid 100 ml.

3. **p-Bromo-aniline reagent.**

   Urinary method. p-Bromo aniline - 2 gm.

   Glacial acetic acid (sat. with thiourea) - 100m

   Blood method. p-Bromo aniline - 5 gm.

   Glacial acetic acid (sat.with thiourea) - 100m

   These solutions are stable for 2 weeks in a dark glass bottle at $4^{0}$C.

4. **Somogyi deproteinising agents.**

   5% Zinc sulphate

   0.3N Barium hydroxide.

   See under Blood sugar determination for details.

Method.

Urine excretion test ( 5 hour and 24 hour collections).

A. 1. Measure volume 5 hour urine and sample 1/10 of this volume.

2. Measure volume subsequent 19 hour urine and sample 1/100 of this.

3. To make up 24 hour aliquot - sample 1/100 of the total 5 hour urine and add to the 1/100 aliquot of the 19 hour urine.

4. Dilute 5 hour and 24 hour aliquots to 1/200 with distilled water.

B. Pipette 1 ml diluted urine into 2 tubes.

C. Pipette 1 ml xylose standard into another 2 tubes.

D. To all 4 tubes add 5 ml of 2% p-Bromo aniline reagent.

E. Mix well and place 1 tube of each pair into 70°C water bath for 10 minutes.

F. Cool tubes to room temperature and place all tubes in the dark for 70 minutes.

G. Read on Klett Colorimeter - Filter 520.

Blood xylose.

1. Capillary blood 0.2 ml from patient is placed into 1.5 ml distilled water.

2. 0.2 ml barium hydroxide solution is added and mixed and without delay 0.2 ml of the zinc sulphate solution is added and mixed.

3. The mixture is allowed to stand for 5 minutes and then centrifuged at 3000 r.p.m. for 5 minutes.

4. 1 ml of the supernatant is then pipetted into another tube.

5. 2 ml of the 5% p-Bromo aniline reagent added and mixed well.

6. Incubated at 70°C for 10 minutes.

7. Cool tubes to room temperature and then place in the dark for 70 minute

8. The control sample consists of 0.2 ml blood drawn from the patient before xylose is administered.
9. Xylose standards are prepared in an identical manner except that 1.5 ml of the xylose standard is substitued for distilled water. Oxalated blood from normal individuals is used in the preparation of the standard as well as for the standard reagent blank against which the standard is read.
10. Read in Klett Colorimeter - 540 filter.

## Blood Sugar Determination.

### Reagents.

1. **Sodium fluoride.**

Stock solution - Sodium fluoride 2.00 gm.
Thymol 0.20 gm.
Potassium oxalate 0.60 gm.
Add water (distilled) to 200 ml.

Working solution - Stock sodium fluoride solution - 8 ml
Add water (distilled) to 420 ml.

2. **Copper reagent.** (Somogyi solutions)

Solution A Potassium sodium tartrate ($KNaC_4H_4O_6\ 4H_2O$) - 12 gm
Sodium carbonate ($Na_2\ CO_3$) anhydrous - 24 gm
Sodium bicarbonate ($Na\ HCO_3$) anhydrous - 16 gm
Sodium sulphate ($Na_2\ SO_4$) anhydrous - 144 gm
Dissolved in water and diluted to 800 ml.

Solution B Copper sulphate ($CuSO_4.5H_2O$) - 4 gm
Sodium sulphate ($Na_2\ SO_4$) anhydrous - 36 gm
Dissolved in water and diluted to 200 ml.

On day of use add 4 parts Sol.A and 1 part Sol.B.

3. **Colour reagent.** (Arseno-molybdic acid)

Ammonium molybdate $(NH_4)_6MO_7O_{24}.\ 4H_2O$ - 25 gm
Dissolve in 450 ml water
Add 21 ml of conc. sulphuric acid ($H_2SO_4$)
Mix
Add sodium arsenate ($Na_2H\ As\ O_4.\ 7H_2O$) - 3 gm dissolved in 25 ml water.

Incubate at 37°C for 24 - 48 hours.

Store in glass stoppered brown bottle.

4. Glucose standards.

a) Glucose 100 mg/100 ml distilled water.

b) Glucose 200 mg/100 ml distilled water.

Keep frozen in deep freeze till used.

5. Somogyi deproteinising agents.

A. 5% Zinc sulphate

Zinc sulphate ($ZnSO_4.7H_2O$) - 25 gm.

Dissolved in water - 500 ml.

B. 0.3N Barium hydroxide (approximate)

Barium hydroxide ($BaCOH)_2.\ 8H_2O$) - 24 gm.

Dissolved in water - 500 ml.

Stored in bottle protected with soda lime.

The barium hydroxide is adjusted in concentration till it requires 4.7 - 4.8 ml to produce a pink colour with phenolthaline when titrated against 5 ml of the zinc sulphate solution.

## Method.

1. In centrifuge tubes.

Into 3.0 ml sodium fluoride working solution wash out 0.2 ml whole blood (capillary).

Protein precipitation.

Add 0.4 ml Barium hydroxide solution.

Shake and wait 10 minutes.

Add 0.4 ml Zinc sulphate solution.

Shake and centrifuge for 20 minutes.

2. Colour development - in Folin-wu tubes.

Supernatant fluid - 1 ml.

Somogyi solutions - 1 ml.

Shake and boil in water bath for 20 minutes.

Cool for 3 - 5 minutes.

Add arsenyl molybdate solution - 1 ml.

Mix and allow to stand for 10 minutes.

Make up to 25 ml mark on tubes with distilled water.

Mix by inverting.

Read in Klett colorimeter with (green) 540 filter.

## Disaccharidase Activity of Duodenal and Jejunal Mucosa.

### Reagents.

1. Normal saline (0.9%)

2. Tris buffer (Sigma)

   Tris buffer 61 gm in 85 ml 5N. hydrochloric acid.

   pH set at 7 - control by adding buffer or HCl.

   Then add water to 1 litre.

3. Glucose standard.

   Glucose 100 mg/1000 ml distilled $H_2O$.

   Keep frozen in deep freeze till used.

4. Glucose reagent.

   Tris buffer (as above) - 1 litre

   Glucose oxidase - 0.125 gm (Fungal Type II - Sigma)

   Peroxidase - 0.02 gm (Horse-radish Type I -Chem. Corp.

   Mix and filter. Keep in refrigerator.

   Working solution - made on day of test.

   100 ml of above filtrate

   0.5 ml ortho-dianisidin solution

   Warm filtrate to $\pm$ 20°C before adding O-dianisidin solution.

5. Ortho-dianisidin solution.

   Ortho-dianisidin - 1 gm
   Abs. alcohol - 100 ml
   } Shake and warm to get solution

6. Substrates. (kept at -4°C) (0.056 M solutions with pH 5.8)

Maltose )
Lactose ) - 200 mg in 10 ml Na. maleate buffer

Sucrose - 190 mg in 10 ml Na. maleate buffer

Sodium maleate buffer 0.1 M, pH 5.8

Stock solutions A) 0.2 M solution of acid sodium maleate.
8 gm of sodium hydroxide + 23.2 gm of maleic acid o
19.6 gm of maleic anhydride in 1000 ml water.

B) 0.2 M sodium hydroxide

50 ml of A. + 20.8 ml of B., diluted to a total of 100 ml with distilled $H_2O$.

During the test equal volumes of substrate and homogenate are used - so the final molarity of the substrate is 0.028 M.

Method.

1. Biopsy wrapped in parafilm and deep frozen until time of analysis.
2. Hopogeniser (Potter-Elvelhjen type)
   Weighed empty
   Weighed with biopsy
   Therefore weight of biopsy known.
3. 0.9% (Normal) saline added. A dilution of 1:20 is required. Add 20 times the weight of saline to biopsy. The biopsy should weigh more than 5 mg and the volume of biopsy + saline should be at least 0.4 ml to start. If the biopsy is too small either a limited range of disaccharidases can be estimated or a greater dilution may be used e.g. 1 : 40 or 1 : 80.
   It should be kept as concentrated as possible.
4. Homogenise - for abour 20 minutes keeping the homogeniser in ice. Homogenise until no particles left.

5. 8 test tubes.

   3 - for incubating substrate with homogenates.

   4 - for substrate + normal saline (substrate blanks).

   1 - for homogennate + normal saline (tissue blank).

6. Homogenate dilution. This varies with expected finding. Whichever activity is thought to be diminished or absent - dilute least.

   This dilution is done with the test tubes kept in ice.

   Homogenate 0.2 ml + 0.2 ml N/saline for lactase and tissue blank.

   0.1 ml of above dilution + 0.3 ml N.saline for sucrase

   0.1 ml of previous dilution + 0.3 ml N.saline for maltase

7. Substrate Blanks.

   | | | |
   |---|---|---|
   | Substrate | - | 50 ɣ |
   | Tris buffer | - | 0.4 ml |
   | N/saline | - | 50 ɣ |

   Substrate placed in bottom of test tube using a micro-pipette

   Added in the above order and then placed in boiling water for 2 minutes

8. Tests and tissue blank.

   Test - diluted homogenate - 50 ɣ

   substrate - 50 ɣ

   Tissue blank - diluted homogenate - 50 ɣ

   N/saline - 50 ɣ

   Incubated for 60 minutes at 37°C. Then removed and placed in ice water

   Add Tris buffer 0.4 ml to these 4 tubes.

   Place in boiling water for 2 minutes to stop reaction.

9. Glucose Standards.

   Blank - 0.5 ml dist. water.

| | | | | | |
|---|---|---|---|---|---|
| Glucose standard | 0.05 ml | + | 0.45 ml | distilled | water |
| | 0.1 ml | + | 0.4 ml | " | " |
| | 0.2 ml | + | 0.3 ml | " | " |
| | 0.3 ml | + | 0.2 ml | " | " |
| | 0.4 ml | + | 0.1 ml | " | " |

10. To measure glucose production.

Add 2 ml glucose reagent (containing oxidase, peroxidase and dianisidin) to all tubes - Tests, substrate blanks, tissue blank and glucose standards.

Incubate all for 60 minutes at 37°C.

11. Read colour change in Spectrophotometer at wave length 436.

## Protein Determination of Biopsies.

### Reagents.

A. 2% Sodium carbonate ($Na_2CO_3$) in 0.1 N sodium hydroxide (NaOH).
0.1 N Sodium hydroxide = 4 gm NaOH in 1000 ml dist. water.

B. 0.5% Copper sulphate ($CuSO_4 . 5H_2O$) in 1% sodium citrate.

C. 1 ml B diluted with A to 50 ml.
Done on day of test as the solution is only stable for a few hours.

D. Folin-Ciocalteus phenol reagent (Merck).
Diluted 1 : 1 with distilled water on day of test.

E. Standard protein
15 mg human albumin in 10 ml 0.9% Sodium chloride.

### Method.

1. Make up protein standards.

| | | | | |
|---|---|---|---|---|
| 1 ml | standard protein | + 6.5 ml | 0.9% NaCl | (0.05 ml = 10 γ) |
| 2 ml | " | " + 4.0 ml | " | (0.05 ml = 25 γ) |
| 2 ml | " | " + 2.0 ml | " | (0.05 ml = 37 γ) |
| 2 ml | " | " + 1.0 ml | " | (0.05 ml = 50 γ) |
| 0.05 ml | " | " - | " | (0.05 ml = 75 γ) |
| | | 0.5 ml | " | (0.05 ml = 0 ) |

2. 0.05 ml protein standards (10 tubes)
0.05 ml saline blank ( 2 tubes)
0.05 ml homogenate ( 1 tube)
i.e. homogenate is not done in duplicate to avoid further dilution.
Dilution of homogenate should be between 1:80 and 1:120.

3. To all tubes.

   2.5 ml reagent C added. Allowed to stand for exactly 10 minutes.

4. To all tubes.

   0.25 ml reagent D added. Shake vigorously. Allow to stand for 2 hours.

Read in photometer.

## Determination of Stool and Dietary Fat.

### Reagents.

1. 33% Potassium hydroxide solution.
2. 96% Ethyl alcohol containing 0.4% amyl alcohol.
3. 25% Hydrochloric acid.
4. Petroleum ether.
5. Thymol blue indicator (2% in 50% ethyl alcohol).
6. N/10 Sodium hydroxide.

### Method.

1. Stool (or feed) thoroughly mixed and 5.91 gm weighed into a 150 ml flask.
2. Add 10 ml 33% potassium hydroxide.
   40 ml 96% ethyl alcohol containing 0.4% amyl alcohol.
3. Boil under reflux condenser for 20 minutes.
4. Cool and add 17 ml 25% hydrochloric acid.
5. Cool and add 50 ml of petroleum ether (at 40-60°C).
6. Shake for 1 minute.
7. Pipette off two amounts of 10 ml of petroleum ether layer.
8. Evaporate off the petroleum ether in a boiling water bath.
9. Add 10 ml 96% ethyl alcohol.
10. Add 2 drops thymol blue indicator.
11. Titrate with N/10 sodium hydroxide.

## Determination of Lactic Acid in Stool.

### Reagents.

A. 20% Copper sulphate ($CuSO_4 . 5 H_2O$) solution.

B. 4% Copper sulphate ($CuSO_4 . 5 H_2O$) solution.

C. 1.5% Solution of p-hydroxy diphenyl in 0.5% sodium hydroxide.

D. Solid calcium hydroxide C.P. (powdered).

E. Concentrated sulphuric acid.

F. Calcium lactate 3.4223 gm per litre distilled water diluted 1/100 so that there is 20 µg/ml as lactic acid.

### Preparation of Stool Sample.

A. Stools weighed and thoroughly blended.

B. 1 gram of stool is diluted with water in a 25 ml volumetric flask.

C. Protein is precipitated by adding trichloro-acetic acid 30% solution until a greater than 7% concentration is obtained. A simple method is to add 2 ml of the stool solution to 3 ml of T.C.A. and 5 ml distilled water. (If expected concentration is low up to 7 ml stool to 3 ml T.C.A. may be used.)

D. Centrifuge for 5 minutes and use 1 ml of supernatant in the following procedure.

### Method.

1. The samples, blank and standard sample should be run in duplicate. The standard sample contains a known amount of lactic acid.

2. 12 ml test tube should be used to afford room to shake the mixture.

3. The blank - contains 9 ml distilled water.
   Standard - contains 5 ml distilled water, + 4 ml of the lactic acid solution (20 µg/ml.)

Stool sample - will contain 1 ml of deproteinised stool + 8 ml of water, (if lactic acid content low may be up to 5 ml of deproteinised stool).

4. To these test tubes add -
   1. 1.0 ml 20% solution of $CuSO_4 . 5 H_2O$.
   2. 1.0 gm calcium hydroxide (this need not be exact).
5. After adding calcium hydroxide.
   1. Shake immediately and vigorously.
   2. Allow tubes to stand at room temperature for half an hour with occasional shaking.
   3. Centrifuge for 5 minutes.
   4. Transfer 1 ml of supernatant to a wide mouthed test tube.
6. Add -
   1. 0.05 ml 4% solution of $CuSO_4 . 5 H_2O$
   2. 6.00 ml concentrated $H_2SO_4$

      Add $H_2SO_4$ slowly from a burette. Add exactly 6.00 ml at a rate of about 1 drop/second. Shake the tube constantly - with bottom of tube resting in cold water bath at about $20^{\circ}C$.
7. Place samples in boiling water for exactly 5 minutes.
8. Cool in cold water bath (below $20^{\circ}C$) for exactly 7 minutes.
9. Add 4 drops p-hydroxy diphenyl solution and shake tubes gently.
10. Allow tubes to stand for 30 minutes in warm water bath about 25 - $30^{\circ}C$. At this point the tubes can be allowed to stand for an extended period without decreasing the yield of lactic acid. The preceeding steps must be carried through without stopping.
11. Place tubes in boiling water for 90 seconds.

12. Cool to room temperature in cold water bath.

13. Read in Klett Colorimeter using 560 filter.

## CHAPTER V.

## RESULTS.


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## CHAPTER V.

## RESULTS.

As stated previously the investigations were performed in two series. The results of the first series led to the design of the experiment and investigations of the second series. The results of the two series are therefore presented separately.

### SERIES I.

The 27 children studied in this series were divided into 2 groups. Group I (16 children) were first given milk feeds and then the carbohydrate-free diet. The second group (11 children) were given the carbohydrate-free diet initially followed by a period on milk.

Group I.

The change from milk to a carbohydrate-free diet was accompanied by a reduction of mean stool weight from 486 gm. to 175 gm. per 24 hours. The mean lactic acid content of the stools showed a similar change. Initially it was high (2.91 gm. per 24 hours) and abruptly fell on the change to a carbohydrate-free diet (0.12 gm. per 24 hours). Chromatography of the stools revealed lactose, glucose and galactose in varying amounts on admission. In only 2 instances were no sugars detected in the stools at this time. As would be expected, the change to a carbohydrate-free diet resulted in the complete disappearance of sugar from the stool. (Table I)

Table I.

Group II.

The change from a carbohydrate-free diet to milk was accompanied by an increase in mean stool weight from 136 gm. to 373 gm. per 24 hours. The mean lactic acid content of the stools showed parallel changes with the stool weights. It rose

## TABLE I

### STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

GROUP I.

| Case No. | Average of 3 days in Grams per 24 hr. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Milk Diet | | | | | Carbohydrate-free diet | | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography G | GA | G |
| 1 | 748 | 4.99 | + | + | + | 71 | 0.03 | - | - | - |
| 2 | 463 | 2.98 | + | + | + | 179 | 0.09 | - | - | - |
| 3 | 441 | 3.57 | - | + | + | 120 | 0.18 | - | - | - |
| 4 | 207 | 0.18 | - | - | - | 229 | 0.04 | - | - | - |
| 5 | 733 | 2.82 | + | + | + | 113 | 0.12 | - | - | - |
| 6 | 793 | 2.98 | + | + | + | 144 | 0.07 | - | - | - |
| 7 | 276 | 0.96 | - | + | - | 244 | 0.09 | - | - | - |
| 8 | 1013 | 14.20 | + | - | - | 175 | 0.37 | - | - | - |
| 9 | 196 | 1.32 | - | + | + | 199 | 0.18 | - | - | - |
| 10 | 185 | 0.36 | - | - | - | 132 | 0.07 | - | - | - |
| 11 | 261 | 0.84 | - | + | - | 160 | 0.09 | - | - | - |
| 12 | 1077 | 7.88 | + | + | + | 332 | 0.13 | - | - | - |
| 13 | 560 | 1.27 | + | + | + | 107 | 0.02 | - | - | - |
| 14 | 128 | 0.82 | + | + | + | 176 | 0.06 | - | - | - |
| 15 | 502 | 1.00 | + | + | + | 218 | 0.26 | - | - | - |
| 16 | 193 | 0.33 | + | + | + | 208 | 0.05 | - | - | - |
| Mean | 486 | 2.91 | | | | 175 | 0.12 | | | |
| S.D. | ±303 | ±3.64 | | | | ±64 | ±0.09 | | | |

Significance : Student (t test)

Stool weight $p < 0.001$

Lactic acid $p < 0.01 > 0.001$

L - Lactose

GA - Galactose

G - Glucose

from 0.07 gm. per 24 hours on the carbohydrate-free diet to 1.49 gm. per 24 hours on milk. Chromatography of the stools on the carbohydrate-free diet showed them to be free of sugar but on changing to milk feeds, 9 of the 11 children had lactose, glucose or galactose, or a combination of these sugars in the stools. (Table II)

Table II.

On examination of the individual results in Groups I and II it was found that they could be each divided into two subgroups on the basis of whether there was a marked change in stool weight on changing the diet or not.

Group I a.

In 10 of the 16 patients in Group I the average daily stool weight fell from 659 gm. per 24 hours to 162 gm. per 24 hours. Mean stool lactic acid was initially 4.25 gm. per 24 hours and fell to 0.14 gm. per 24 hours. (Table III)

Table III.

Group I b.

The other 6 patients in Group I did not have a dramatic response on changing the diet. Initially these children did not have severe diarrhoea, the average daily stool weight on milk being 198 gm. per 24 hours. The mean stool lactic acid content was not grossly elevated being 0.66 gm. per 24 hours. There was no change in stool weight on being put on the carbohydrate-free diet and although the stool lactic acid content dropped to 0.08 gm. per 24 hours this difference is not statistically significant. (Table IV)

Table IV.

Group II a.

In 6 patients of Group II marked diarrhoea occurred on changing from the carbohydrate-free diet to milk. The mean stool weight rose from 167 gm. to 581 gm. per 24 hours. Stool lactic acid content rose in parallel from 0.11 gm. to 2.5 gm. per 24 hours. (Table V)

TABLE II.

STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

GROUP II.

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbohydrate-free Diet | | | | | Milk | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 17 | 263 | 0.09 | - | - | - | 158 | 0.34 | - | + | + |
| 18 | 193 | 0.03 | - | - | - | 492 | 0.80 | + | + | + |
| 19 | 290 | 0.17 | - | - | - | 711 | 2.52 | + | + | + |
| 20 | 105 | 0.06 | - | - | - | 337 | 1.72 | - | + | + |
| 21 | 126 | 0.02 | - | - | - | 188 | 0.64 | - | - | - |
| 22 | 33 | 0.01 | - | - | - | 84 | 0.15 | - | - | + |
| 23 | 18 | 0.00 | - | - | - | 63 | 0.05 | - | - | - |
| 24 | 55 | 0.02 | - | - | - | 122 | 0.21 | - | + | - |
| 25 | 135 | 0.18 | - | - | - | 1172 | 6.48 | - | + | + |
| 26 | 198 | 0.08 | - | - | - | 527 | 2.38 | - | + | + |
| 27 | 81 | 0.12 | - | - | - | 246 | 1.09 | + | + | tr. |
| Mean | 136 | 0.07 | | | | 373 | 1.49 | | | |
| S.D. | ±91 | ±0.06 | | | | ±336 | ±1.87 | | | |

Significance : Student (t test)

Stool weight $p < 0.05 > 0.02$

Lactic acid $p < 0.02 > 0.01$

## TABLE III.

## STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

### GROUP I a

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Milk | | | | | Carbohydrate-free Diet | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 1 | 748 | 4.99 | + | + | + | 71 | 0.03 | - | - | - |
| 2. | 463 | 2.98 | + | + | + | 179 | 0.09 | - | - | - |
| 3 | 441 | 3.57 | - | + | + | 120 | 0.18 | - | - | - |
| 5 | 733 | 2.82 | + | + | + | 113 | 0.12 | - | - | - |
| 6 | 793 | 2.98 | + | + | + | 144 | 0.07 | - | - | - |
| 8 | 1013 | 14.20 | + | - | - | 175 | 0.37 | - | - | - |
| 11 | 261 | 0.84 | - | + | - | 160 | 0.09 | - | - | - |
| 12 | 1077 | 7.88 | + | + | + | 332 | 0.13 | - | - | - |
| 13 | 560 | 1.27 | + | + | + | 107 | 0.02 | - | - | - |
| 15 | 502 | 1.00 | + | + | + | 218 | 0.26 | - | - | - |
| Mean | 659 | 4.25 | | | | 162 | 0.14 | | | |
| S.D. | ±260 | ±4.08 | | | | ±73 | ±0.11 | | | |

Significance : Student (t test)

Stool weight $p < 0.001$

Lactic acid $p < 0.01 > 0.001$

## TABLE IV.

## STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

### GROUP I b.

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Milk Diet | | | | | Carbohydrate-free Diet | | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 4 | 207 | 0.18 | - | - | - | 229 | 0.04 | - | - | - |
| 7 | 276 | 0.96 | - | + | - | 244 | 0.09 | - | - | - |
| 9 | 196 | 1.32 | - | + | + | 199 | 0.18 | - | - | - |
| 10 | 185 | 0.36 | - | - | - | 132 | 0.07 | - | - | - |
| 14 | 128 | 0.82 | + | + | + | 176 | 0.06 | - | - | - |
| 16 | 193 | 0.33 | + | + | + | 208 | 0.05 | - | - | - |
| Mean | 198 | 0.66 | | | | 198 | 0.08 | | | |
| S.D. | ±48 | ±0.44 | | | | ±40 | ±0.05 | | | |

Significance : Student (t test)

Stool weights : not significant

Lactic acid : not significant

TABLE V.

STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

GROUP II a.

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbohydrate-free Diet | | | | | Milk Diet | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 18 | 193 | 0.03 | - | - | - | 492 | 0.80 | + | + | + |
| 19 | 290 | 0.17 | - | - | - | 711 | 2.52 | + | + | + |
| 20 | 105 | 0.06 | - | - | - | 337 | 1.72 | - | + | + |
| 25 | 135 | 0.18 | - | - | - | 1172 | 6.48 | - | + | + |
| 26 | 198 | 0.08 | - | - | - | 527 | 2.38 | - | + | + |
| 27 | 81 | 0.12 | - | - | - | 246 | 1.09 | + | + | + |
| Mean | 167 | 0.11 | | | | 581 | 2.50 | | | |
| S.D. | ±76 | ±0.06 | | | | ±331 | ±2.07 | | | |

Significance : Student (t test)

Stool weight $p < 0.02 > 0.01$

Lactic acid $p < 0.02 > 0.01$

Group II b.

The other 5 children in Group II showed little change on being fed milk. The milk did not induce a severe diarrhoea. Stool weight rose from 99 gm. to 123 gm. per 24 hours and lactic acid content from 0.03 gm. to 0.28 gm. per 24 hours. (Table VI)

Table VI.

Group I and II.

When Groups I and II are considered together, mean stool weight and lactic acid content on the milk diet was 440 gm. and 2.33 gm. per 24 hours respectively. On the carbohydrate-free diet stool weight and lactic acid content were lower being 159 gm. and 0.09 gm. per 24 hours. Sixteen of the 27 children (59 per cent) (Groups I a and II a) had marked differences on changing from milk to a carbohydrate-free diet. Mean stool weight dropped from 630 gm. to 164 gm. per 24 hours and mean stool lactic acid content from 3.59 gm. to 0.13 gm. per 24 hours. In the other 11 children (Groups I b and II b) the change of diet had no significant effect on the stool weight and lactic acid content. (Table VII)

Table VII.

Clinical features of the children in Series I.

The clinical features of the 27 children included in this series are summarized in Table VIII.

Table VIII.

The mean age was 1 year 10 months with a range from 8 months to 4 years 9 months. The average weight was 7.7 kg. The mean per cent of expected weight was 63% of the 50th percentile of the Boston Children's Medical Center percentile chart. Mean serum protein concentration on admission was 3.66 gm. per 100 ml with a serum albumin concentration of 1.55 gm% (range 0.8 - 2.54 gm%). Mean haemoglobin concentration was 9.6 gm%.

## TABLE VI.

## STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

### GROUP II b.

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbohydrate-free Diet | | | | | Milk Diet | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 17 | 263 | 0.09 | - | - | - | 158 | 0.34 | - | + | + |
| 21 | 126 | 0.02 | - | - | - | 188 | 0.64 | - | - | - |
| 22 | 33 | 0.01 | - | - | - | 84 | 0.15 | - | - | + |
| 23 | 18 | 0.00 | - | - | - | 63 | 0.05 | - | - | - |
| 24 | 55 | 0.02 | - | - | - | 122 | 0.21 | - | + | - |
| Mean | 99 | 0.03 | | | | 123 | 0.28 | | | |
| S.D. | ±101 | ±0.04 | | | | ±51 | ±0.23 | | | |

Significance : Student (t test)

Stool weights : not significant

Lactic acid : not significant

## TABLE VII.

## STOOL WEIGHTS AND LACTIC ACID CONTENT.

### SERIES I.

| Group | No. of Cases | Average of means of 3 days and S.D. in Grams per 24 hrs. | | | Significance (p) |
|---|---|---|---|---|---|
| | | | Milk Diet | Carbohydrate-free Diet | |
| I & II | 27 | Stool weight | 440 (±318) | 159 (±76) | < 0.001 |
| | | Lactic acid | 2.33 (±3.08) | 0.09 (±0.08) | < 0.001 |
| Ia & IIa | 16 | Stool weight | 630 (±281) | 164 (±72) | < 0.001 |
| | | Lactic acid | 3.59 (±3.47) | 0.13 (±0.09) | < 0.001 |
| Ib & IIb | 11 | Stool weight | 164 (±61) | 153 (±86) | N.S. |
| | | Lactic acid | 0.49(±0.04) | 0.05 (±0.05) | N.S. |

N.S. - not significant

## TABLE VIII.

## CLINICAL FEATURES.

## SERIES I.

| Case No. | Age yr/mth | Wt (kg) | % Exp. WT. | Serum Proteins | | Haematology | |
|---|---|---|---|---|---|---|---|
| | | | | Total | Albumin | Hb. | P.C.V. |
| 1 | 1/6 | 7.94 | 70 | 3.84 | 1.67 | 9.6 | 28 |
| 2 | 1/6 | 9.09 | 80 | 3.19 | 1.29 | 9.2 | 28 |
| 3 | 1/9 | 7.84 | 66 | 2.77 | 1.18 | 10.4 | 30 |
| 4 | 1/11 | 6.62 | 54 | 3.31 | 1.64 | 10.1 | 30 |
| 5 | 1/5 | 8.27 | 75 | 2.76 | 1.28 | 10.0 | 31 |
| 6 | 1/0 | 7.04 | 71 | 3.36 | 1.42 | 9.8 | 28 |
| 7 | 0/8 | 5.15 | 60 | 3.98 | 1.28 | 10.9 | 32 |
| 8 | 1/10 | 7.98 | 67 | 3.56 | 1.75 | 9.3 | 28 |
| 9 | 1/3 | 6.82 | 64 | 3.55 | 1.25 | 9.5 | 27 |
| 10 | 0/9 | 5.65 | 63 | 3.23 | 1.26 | 11.6 | 41 |
| 11 | 1/8 | 9.88 | 84 | 2.35 | 1.11 | 11.5 | 36 |
| 12 | 3/10 | 13.72 | 87 | 3.27 | 1.11 | 9.9 | 31 |
| 13 | 2/7 | 6.17 | 46 | 3.33 | 1.71 | 11.6 | 36 |
| 14 | 1/5 | 6.73 | 61 | 2.79 | 1.02 | 7.6 | 26 |
| 15 | 1/5 | 5.71 | 51 | 4.30 | 2.22 | 10.1 | 30 |
| 16 | 1/9 | 8.43 | 71 | 3.52 | 1.33 | 9.4 | 26 |
| 17 | 1/10 | 6.05 | 50 | 4.03 | 1.68 | 9.2 | 31 |
| 18 | 1/5 | 5.29 | 48 | 3.33 | 2.03 | 9.0 | 32 |
| 19 | 2/5 | 5.47 | 41 | 4.45 | 2.15 | 7.2 | 26 |
| 20 | 1/4 | 7.04 | 65 | 2.81 | 0.80 | 8.2 | 29 |
| 21 | 4/9 | 11.23 | 65 | 4.06 | 1.49 | 8.8 | 31 |
| 22 | 1/0 | 5.99 | 60 | 4.94 | 2.45 | 11.1 | 33 |
| 23 | 1/9 | 7.32 | 62 | 4.75 | 1.71 | 7.5 | 24 |
| 24 | 4/8 | 11.84 | 69 | 6.17 | 2.54 | 10.7 | 33 |
| 25 | 1/8 | 10.69 | 91 | 3.71 | 1.43 | 11.5 | 31 |
| 26 | 1/3 | 7.50 | 71 | 4.25 | 1.58 | 7.0 | 14 |
| 27 | 1/6 | 6.49 | 57 | 3.34 | 1.35 | 10.0 | 33 |
| Mean | 1/10 | 7.7 | 65 | 3.66 | 1.55 | 9.6 | 29 |

On the basis of the marked difference that occurred on changing from milk to the carbohydrate-free diet (or vice versa) Groups Ia and IIa were considered carbohydrate intolerant. The clinical features of these patients are summarised in Table IX.

Table IX.

The mean age was 1 year 9 months with a range from 1 year to 3 years 10 months. The average weight was 7.9 kg. with a mean per cent of expected weight of 67. On admission mean serum protein concentration was 3.41 gm% (globulin 1.91 gm% and albumin 1.51 gm%). Mean haemoglobin concentration was 9.6 gm%.

By the same criteria Ib and IIb were considered to be tolerant of carbohydrate. The clinical features of these patients are summarised in Table IX. The mean age was 2 years with a range from 8 months to 4 years 8 months. The mean weight was 7.4 kg. and were on average 62% of expected weight. The mean serum protein concentration on admission was 4.03 gm% with a serum albumin concentration of 1.6 gm%. Mean haemoglobin concentration was 9.6 gm%.

There is no significant difference in these parameters between the carbohydrate intolerant and non-carbohydrate intolerant groups. No other differences were noted on clinical grounds.

Absorption Tests:

D-xylose absorption test: In the age group of the children under study the normal 5 hour urinary excretion of xylose was conservatively taken as greater than 14 per cent of the ingested dose.[181-183] When on milk diet 3 of the 6 children studied (2 with kwashiorkor and 1 with marasmus) had impaired absorption of xylose. (Table X)

Table X.

The percentage of ingested xylose excreted was 7.1, 5.6, and 6.6 respectively. After control of the diarrhoea with the carbohydrate-free diet, the percentage of xylose excreted had improved in all 6 children, although one was still abnormal. In this

## TABLE IX.

## COMPARISON CLINICAL FEATURES.

### SERIES I.

| Group | Age yr/mth. | Wt. (kg) | %Exp. Wt. | Serum Proteins | | Haematology | |
|---|---|---|---|---|---|---|---|
| | | | | Total | Albumin | Hb. | P.C.V. |
| Ia and IIa | 1/9 | 7.88 | 67 | 3.41 | 1.51 | 9.6 | 29 |
| Ib and IIb | 2/0 | 7.44 | 62 | 4.03 | 1.60 | 9.7 | 30 |

TABLE X.

PERCENTAGE URINARY XYLOSE RECOVERY.

| Case No. | Milk Diet | Carbohydrate-free Diet |
|---|---|---|
| 10 | 13.6 | 20 |
| 11 | 7.1 | 14.5 |
| 12 | 5.6 | 33.7 |
| 13 | 6.6 | 10.8 |
| 14 | 23.7 | 24.0 |
| 16 | 14.6 | 18.5 |
| Mean | 11.9 | 19.5 |

child the xylose excretion rose from 6.6 to 10.8 per cent. There was no correlation between xylose excreted and the severity of the initial diarrhoea in this small number of patients.

Carbohydrate Tolerance Tests: In 3 of the 5 children in whom oral mono- and disaccharide tolerance tests were carried out, the diarrhoea had been significantly improved by the change to a carbohydrate-free diet. Two were children with nutritional marasmus, and one had kwashiorkor. Oral lactose loads in these 3 children produced a much smaller blood sugar increment than that which occurred after loading with its constituent monosaccharides glucose and galactose (Fig. 2, A, B and C). The average stool weight prior to the lactose load was 183 gm. per 24 hours, and following the test, 400 gm. per 24 hours. Lactic acid content of the stool also increased, and glucose and galactose were recovered from the stool by chromatography.

In one child with nutritional marasmus, lactose tolerance tests were performed at intervals for 7 months after admission. The blood sugar curve remained flat and each tolerance test was followed by an episode of diarrhoea. Seven months after admission glucose/galactose, sucrose and maltose loads were followed by a satisfactory increase in blood sugar and no diarrhoea. A lactose load produced only a small transient blood sugar increment and was followed by a brief episode of diarrhoea. (Fig. 2, C and D)

In the 2 children (both with kwashiorkor) who did not initially have very severe diarrhoea and in whom a carbohydrate-free diet did not produce marked improvement, a lactose load gave comparable increments of blood sugar to a glucose/galactose mixture. (Fig.3) No diarrhoea occurred following the lactose load.

Bacteriology:

In 2 children Salmonellae were isolated, 2 had Shigellae and 4 had Giardia lamblia. All of these children had large stool weights and elevated stool lactic acid content while on milk. All responded immediately to the removal of carbohydrate from

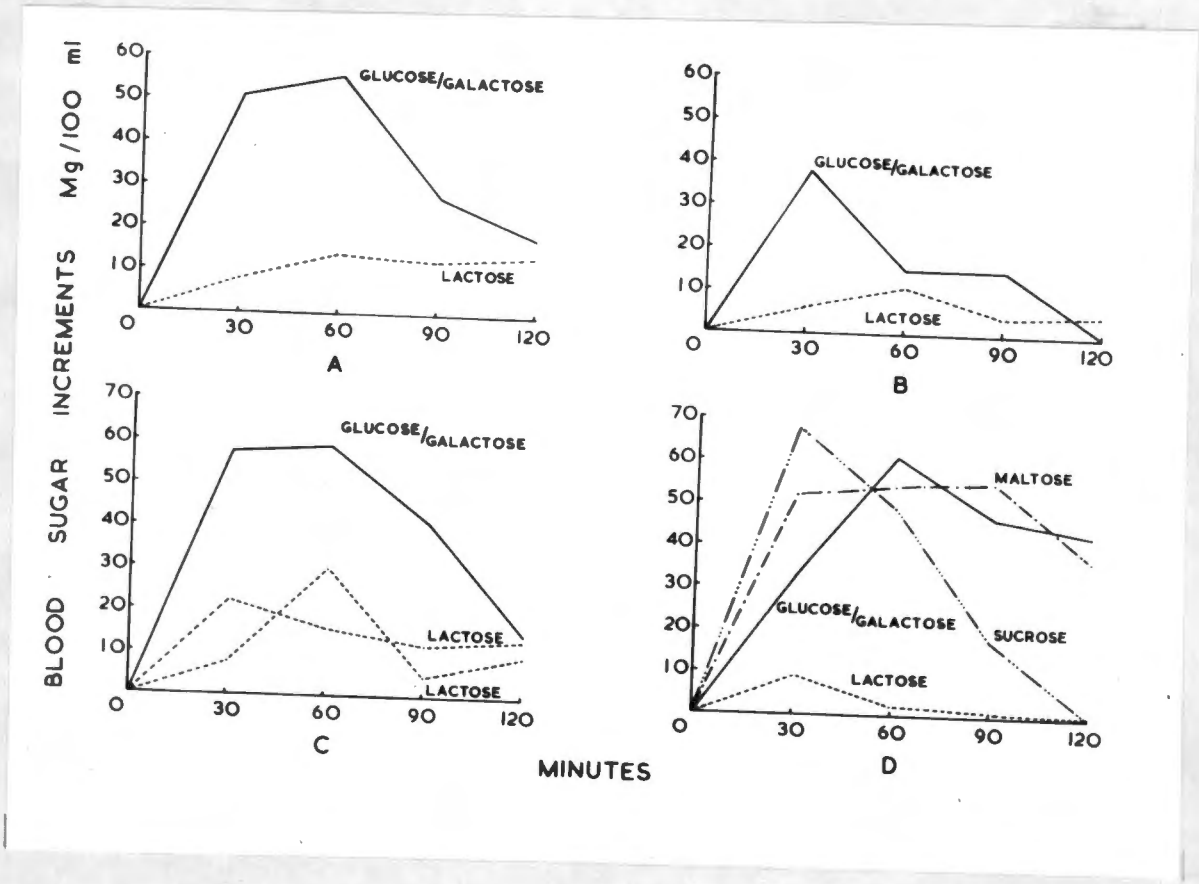


**FIGURE 2.**

Carbohydrate tolerance tests in 3 of the children in whom the diarrhoea was controlled by the carbohydrate-free diet. A,B, C, Blood sugar increments following administration of glucose/ galactose and lactose within 10 days of admission. D, Same child as in C; mono - and disaccharide loads given 7 months after admission.

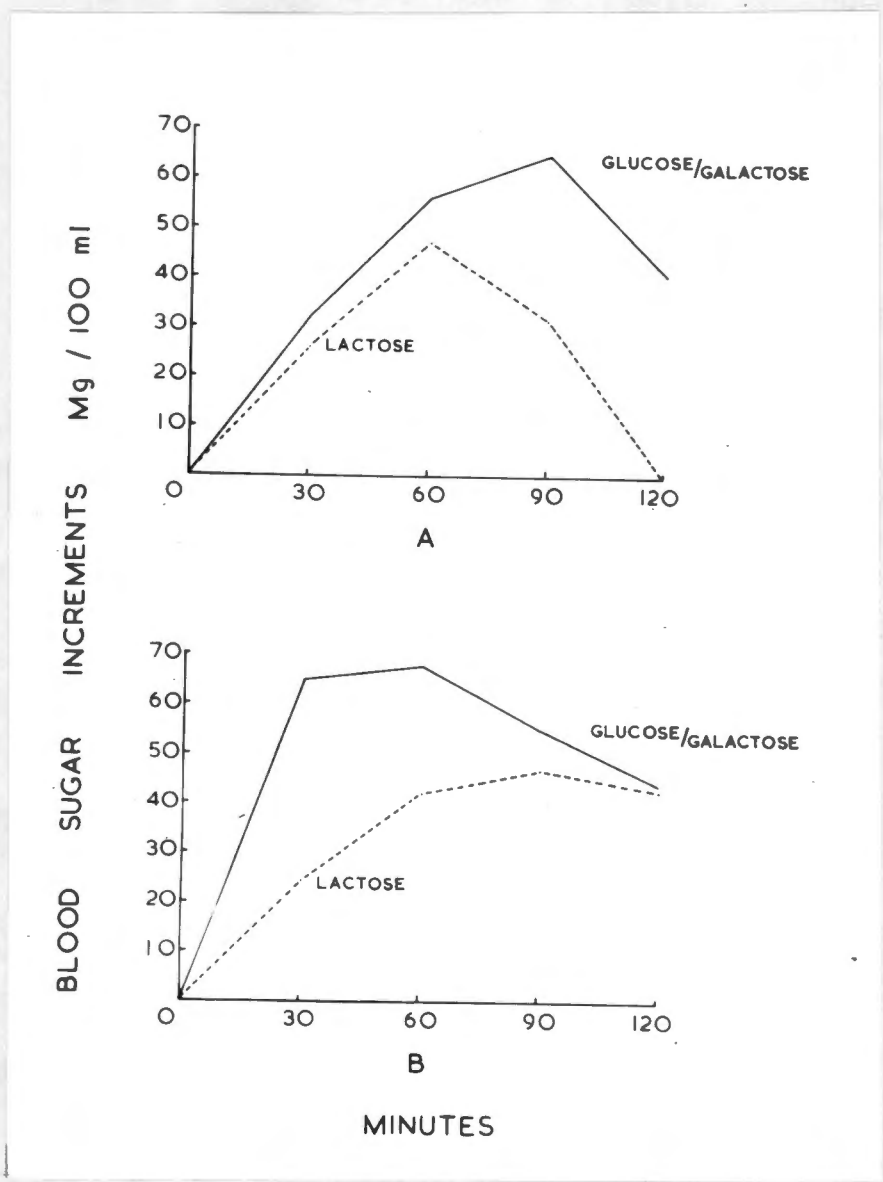


**FIGURE 3.**

**Two children who did not have severe diarrhoea and whose diarrhoea was not improved by the carbohydrate-free diet. Blood sugar increments following glucose/galactose and lactose loads.**

the diet. Thus, in 8 of the 16 children (Groups Ia and IIa) with severe diarrhoea, recognised intestinal pathogens were isolated from the stools. No pathogens were isolated from the stools of any of the 11 children (Groups Ib and IIb) who did not have severe diarrhoea while under observation.

SERIES II.

All twenty children in this series were studied in an identical manner.

Stools:

The change from a disaccharide (lactose) containing diet (milk) to a diet containing monosaccharide only (Disaccharide-free diet) was accompanied by a fall in stool weight and a drop in the stool lactic acid content. Conversely when these children were put back on the lactose containing diet the stool weight and lactic acid content per 24 hours rose again. The sugar content of the stools showed similar variations. While on the initial milk diet, 17 of the 20 children had lactose or its constituent monosaccharides present in the stools. On the disaccharide-free diet only trace amounts of sugar were detected in a few stools but when the children were put back on milk 15 of the 20 again had significant amounts of sugar in the stools. (Table XI)

Table XI.

Closer scrutiny of these results show two groups of cases. Thirteen children showed marked variations of stool weight, stool lactic acid content, and stool sugar on being changed from milk to a disaccharide-free diet and back again. In this group (designated "non-absorbers") mean stool weights fell from 506 to 123 gm. and then rose again to 430 gm. per 24 hours on reintroduction of milk. Stool lactic acid content showed parallel changes. All stools contained measurable quantities of lactose, galactose, or glucose on milk but only traces of these sugars were found on the disaccharide-free diet. (Table XII)

Table XII.

## TABLE XI.

STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

### SERIES II.

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Milk Diet | | | | | Disaccharide-free Diet | | | | | Milk Diet | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 1 | 99 | 0.13 | + | + | + | 8 | 0.01 | - | - | - | 74 | 0.11 | - | - | - |
| 2 | 352 | 0.29 | - | + | - | 171 | 0.08 | - | - | - | 194 | 0.32 | - | - | - |
| 3 | 106 | 0.07 | + | + | + | 97 | 0.01 | - | tr | - | 45 | 0.25 | - | + | + |
| 4 | 188 | 0.39 | + | + | + | 77 | 0.03 | - | - | - | 86 | 0.14 | - | + | + |
| 5 | 123 | 0.36 | - | + | - | 34 | 0.01 | - | - | - | 141 | 0.34 | - | + | + |
| 6 | 212 | 0.94 | + | + | + | 99 | 0.01 | - | + | tr | 503 | 1.99 | + | + | + |
| 7 | 225 | 0.10 | + | + | tr | 90 | 0.01 | - | - | - | 209 | 0.33 | - | + | + |
| 8 | 323 | 2.53 | tr | + | + | 76 | 0.15 | - | - | - | 358 | 4.15 | + | + | + |
| 9 | 450 | 3.79 | + | + | + | 106 | 0.10 | - | - | - | 475 | 4.44 | - | + | + |
| 10 | 333 | 1.90 | + | + | + | 110 | 0.22 | - | tr | tr | 418 | 3.52 | + | + | + |
| 11 | | | tr | + | tr | | | | | | | | - | + | + |
| 12 | 315 | 0.98 | + | + | + | 138 | 0.58 | tr | - | - | 579 | 1.82 | + | + | + |
| 13 | 86 | 0.36 | - | - | - | 83 | 0.32 | - | - | - | | | | | |
| 14 | 71 | 0.06 | - | - | - | 42 | 0.03 | - | - | - | 71 | 0.06 | - | - | - |
| 15 | 132 | 0.21 | - | - | - | 97 | 0.04 | - | - | - | 99 | 0.48 | - | - | - |
| 16 | 449 | 2.85 | - | + | + | 60 | 0.32 | - | tr | - | 362 | 2.22 | - | + | + |
| 17 | 263 | 1.69 | + | + | + | 104 | 0.03 | - | - | - | 210 | 0.91 | - | + | + |
| 18 | 2045 | 10.04 | + | + | + | 305 | 0.17 | - | tr | - | 652 | 4.37 | tr | + | + |
| 19 | 440 | 0.43 | + | + | + | 122 | 0.02 | - | - | - | 502 | 3.85 | + | + | + |
| 20 | 891 | 2.89 | + | + | + | 233 | 0.08 | - | - | - | 755 | 4.48 | + | + | + |
| Mean | 374 | 1.58 | | | | 108 | 0.12 | | | | 319 | 1.88 | | | |
| S.D. | ±449 | ±2.35 | | | | ±69 | ±0.15 | | | | ±223 | ±1.78 | | | |

**Significance** : Student (t test)

**Stool weights** 374/108 - $p < 0.02$
108/319 - $p < 0.001$

**Lactic acid** 1.58/0.12 - $p < 0.02$
0.12/1.88 - $p < 0.001$

L - Lactose
GA - Galactose
G - Glucose

## TABLE XII.

STOOL WEIGHTS, LACTIC ACID CONTENT, AND CHROMATOGRAPHY FOR SUGARS.

NON-ABSORBERS.

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Milk Diet | | | | | Disaccharide-free Diet | | | | | Milk Diet | | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 5 | 123 | 0.36 | - | + | - | 34 | 0.01 | - | - | - | 141 | 0.34 | - | + | + |
| 6 | 212 | 0.94 | + | + | + | 99 | 0.01 | - | + | tr | 503 | 1.99 | + | + | + |
| 7 | 225 | 0.10 | + | + | tr | 90 | 0.01 | - | - | - | 209 | 0.33 | - | + | + |
| 8 | 323 | 2.53 | tr | + | + | 76 | 0.15 | - | - | - | 358 | 4.15 | + | + | + |
| 9 | 450 | 3.79 | + | + | + | 106 | 0.10 | - | - | - | 475 | 4.44 | - | + | + |
| 10 | 333 | 1.90 | + | + | + | 110 | 0.22 | - | tr | tr | 418 | 3.52 | + | + | + |
| 11 | | | tr | + | tr | | | | | | | | | | |
| 12 | 315 | 0.98 | + | + | + | 138 | 0.58 | tr | - | - | 579 | 1.82 | + | + | + |
| 16 | 449 | 2.85 | - | + | + | 60 | 0.32 | - | tr | - | 362 | 2.22 | - | + | + |
| 17 | 263 | 1.69 | + | + | + | 104 | 0.03 | - | - | - | 210 | 0.91 | - | + | + |
| 18 | 2045 | 10.04 | + | + | + | 305 | 0.47 | - | tr | - | 652 | 4.37 | tr | + | + |
| 19 | 440 | 0.43 | + | + | + | 122 | 0.02 | - | - | - | 502 | 3.85 | + | + | + |
| 20 | 891 | 2.89 | + | + | + | 233 | 0.08 | - | - | - | 755 | 4.48 | + | + | + |
| Mean | 506 | 2.38 | | | | 123 | 0.14 | | | | 430 | 2.70 | | | |
| S.D. | ±522 | ±2.68 | | | | ±75 | ±0.17 | | | | ±186 | ±1.62 | | | |

Significance : Student (t test)

Stool weights 506/123 - $p < 0.02$
123/430 - $p < 0.001$

Lactic acid 2.38/0.14 - $p < 0.01$
0.14/2.70 - $p < 0.001$

In seven children ("the absorbers") there was little variation in mean stool weight and stool lactic acid content on changing the diets. In four sugar was present in the stool initially, only traces were detected on the disaccharide-free diet, and in only two did sugar reappear in the stool when milk was reintroduced. (Table XIII)

Table XIII.

Absorption Tests:

Xylose:

No significant change of the urinary excretion of xylose (5 and 24 hours) was noted during the three diet periods. The mean 5 hour excretion of xylose was 25 - 26% and the mean 24 hour excretion 36 - 38% of the ingested dose for the three periods. (Table XIV and Fig.4)

Table XIV.

The difference between 5 and 24 hour excretion was statistically significant for all three tests (Student (t test) $p < 0.02$, 0.05,and 0.05 respectively). A greater number of very low values was found in the 5 hour than in the 24 hour excretion test.(Fig.4) Only one patient had low values (7 and 9%, respectively) for both 5 and 24 hour excretion. This test was done during the initial period on milk when the child was passing over 2,000 gm. of stool per 24 hours. There was no significant difference in the 5 and 24 hour excretion of xylose in comparing absorbers and non-absorbers.

Blood xylose levels in the first 2 hours after ingestion were very similar during all three test periods. (Table XV and Fig.5)

Table XV.

The highest mean blood xylose levels were between 28 and 31 mg./100 ml and occurred at 120 minutes after ingestion.

## TABLE XIII.

## STOOL WEIGHTS, LACTIC ACID CONTENT AND CHROMATOGRAPHY FOR SUGARS.

### ABSORBERS

| Case No. | Average of 3 days in Grams per 24 hrs. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Milk Diet | | | | | Disaccharide-free Diet | | | | | Milk Diet | | | | |
| | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G | Stool Wt. | Lactic Acid | Chromatography L | GA | G |
| 1 | 99 | 0.13 | + | + | + | 8 | 0.01 | - | - | - | 74 | 0.11 | - | - | - |
| 2 | 352 | 0.29 | - | + | - | 171 | 0.08 | - | - | - | 194 | 0.32 | - | - | - |
| 3 | 106 | 0.07 | + | + | + | 97 | 0.01 | - | tr | - | 45 | 0.25 | - | + | + |
| 4 | 188 | 0.39 | + | + | + | 77 | 0.03 | - | - | - | 86 | 0.14 | - | + | + |
| 13 | 86 | 0.36 | - | - | - | 83 | 0.32 | - | - | - | | | | | |
| 14 | 71 | 0.06 | - | - | - | 42 | 0.03 | - | - | - | 71 | 0.06 | - | - | - |
| 15 | 132 | 0.21 | - | - | - | 97 | 0.04 | - | - | - | 99 | 0.48 | - | - | - |
| Mean | 148 | 0.22 | | | | 82 | 0.07 | | | | 95 | 0.23 | | | |
| S.D. | ±98 | ±0.14 | | | | ±51 | ±0.11 | | | | ±52 | ±0.16 | | | |

Significance : Student (t test)

Stool weights 148/82 - not significant
82/95 - not significant
Lactic acid 0.22/0.07 - $p < 0.05$
0.07/0.23 - not significant

## TABLE XIV.

### % URINARY XYLOSE RECOVERIES.

| Case No. | 1st TEST Milk Diet | | 2nd TEST Disaccharide-free Diet | | 3rd TEST Milk Diet | |
|---|---|---|---|---|---|---|
| | 5 hr. | 24 hr. | 5 hr. | 24 hr. | 5 hr. | 24 hr. |
| 1 | 16 | 30 | 22 | 30 | 28 | 36 |
| 2 | 21 | 49 | 34 | 39 | 36 | 47 |
| 3 | 9 | 43 | 2 | 17 | 9 | 20 |
| 4 | 48 | 56 | 49 | 49 | | |
| 5 | 36 | | 27 | | 19 | |
| 6 | 60 | 91 | 36 | 72 | 50 | 68 |
| 7 | 36 | 31 | 60 | 55 | 29 | 37 |
| 8 | 14 | 23 | 11 | 11 | 3 | 58 |
| 9 | 3 | 26 | | | 22 | 28 |
| 10 | 35 | 43 | 16 | 47 | 33 | 41 |
| 11 | 19 | 31 | 22 | 37 | 18 | 22 |
| 12 | 13 | 21 | 22 | 26 | | |
| 13 | 25 | 37 | 12 | 30 | | |
| 14 | 21 | 27 | 23 | 32 | 23 | 27 |
| 15 | 20 | 28 | 16 | 26 | 24 | 22 |
| 16 | 28 | 54 | 32 | 55 | 29 | 38 |
| 18 | 7 | 9 | 9 | 17 | 3 | 23 |
| 19 | 24 | 49 | 37 | 44 | 22 | 25 |
| 20 | 31 | 37 | 42 | 59 | 55 | 48 |
| Mean | 25 | 38 | 26 | 38 | 25 | 36 |
| S.D. | ±14 | ±18 | ±15 | ±17 | ±14 | ±14 |

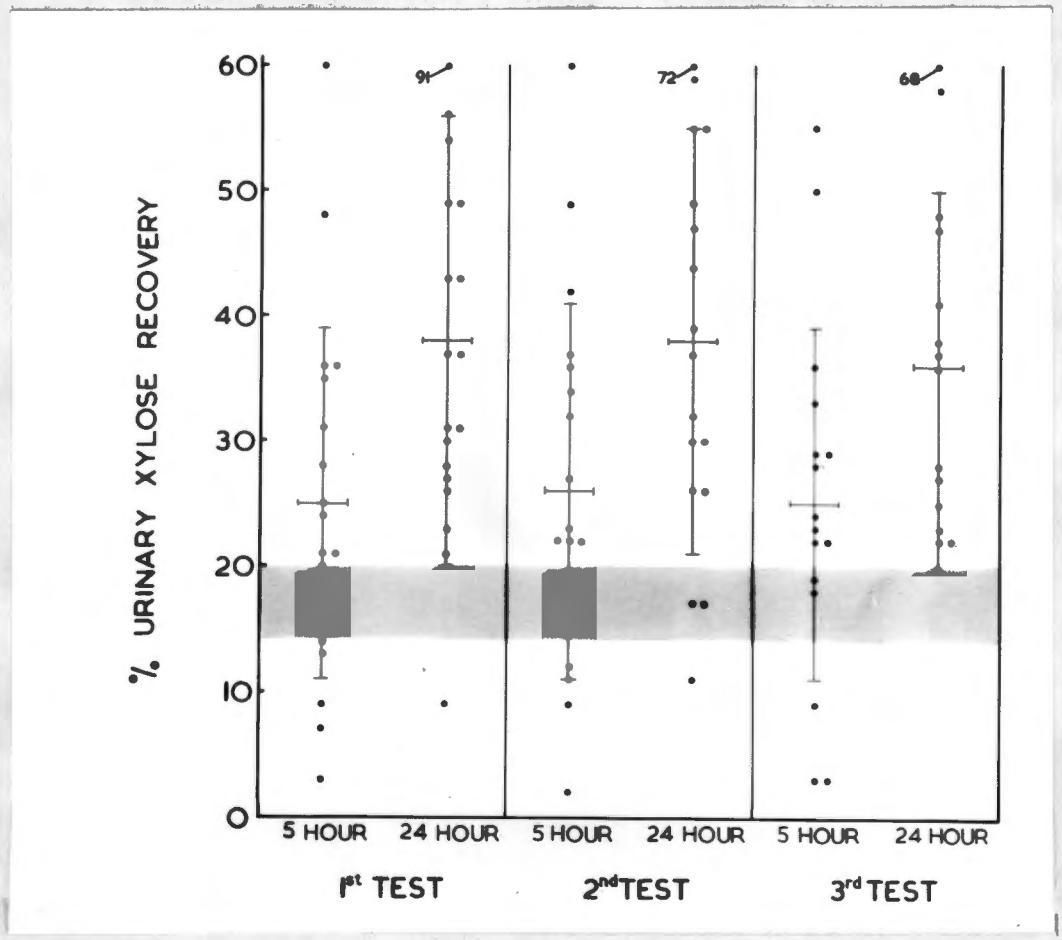


**FIGURE 4.**

**Five and 24 hour urinary xylose recoveries during the three tests. Horizontal bars represent the means and the vertical bars 1 SD above and below the means. Shaded strip represents the lowest normal 5 hour xylose recoveries reported by various authors.**

## TABLE XV.

## BLOOD XYLOSE LEVELS.

| Case NO. | 1st Test Milk Diet | | | | 2nd Test Disaccharide-free Diet | | | | 3rd Test Milk Diet | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Minutes | | | | Minutes | | | | Minutes | | | |
| | 30 | 60 | 90 | 120 | 30 | 60 | 90 | 120 | 30 | 60 | 90 | 120 |
| 1 | 15 | 23 | 27 | 31 | 13 | 22 | 22 | 26 | 20 | 22 | 28 | 30 |
| 2 | 20 | 23 | 24 | 23 | 9 | 20 | 22 | 24 | 24 | 23 | 30 | 29 |
| 3 | 19 | 47 | 26 | 35 | 2 | 2 | 5 | 6 | | | | |
| 4 | 21 | 35 | 40 | 39 | 24 | 35 | 38 | 41 | | | | |
| 8 | | | | | 1 | 6 | 8 | 15 | | | | |
| 9 | | | | | | | | | 14 | 18 | 27 | 28 |
| 10 | | | | | 8 | 16 | 21 | 24 | 20 | 23 | 29 | 35 |
| 11 | 3 | 14 | 31 | 30 | | | | | 1 | 8 | 16 | 18 |
| 12 | 1 | 3 | 8 | 15 | 8 | 16 | 17 | 17 | | | | |
| 13 | 11 | 18 | 27 | 31 | 9 | 26 | 35 | 35 | | | | |
| 14 | 4 | 23 | 34 | 35 | 5 | 14 | 25 | 29 | 4 | 14 | 20 | 24 |
| 15 | 24 | 32 | 28 | 29 | | | | | 17 | 29 | 26 | 21 |
| 16 | 11 | 31 | 49 | 37 | 25 | 31 | 41 | 42 | 14 | 34 | 46 | 38 |
| 18 | 0 | 2 | 8 | 12 | | | | | 5 | 16 | 16 | 17 |
| 19 | 13 | 25 | 35 | 32 | 30 | 35 | 40 | 40 | 15 | 20 | 24 | 18 |
| 20 | 18 | 32 | 39 | 39 | 16 | 20 | 29 | 32 | 22 | 38 | 45 | 37 |
| Mean | 12 | 24 | 29 | 31 | 13 | 20 | 25 | 28 | 14 | 22 | 28 | 27 |
| S.D. | ±8.1 | ±12.6 | ±11.6 | ±9.0 | ±9.4 | ±10.4 | ±11.9 | ±11.2 | ±7.7 | ±8.8 | ±9.9 | ±7.8 |

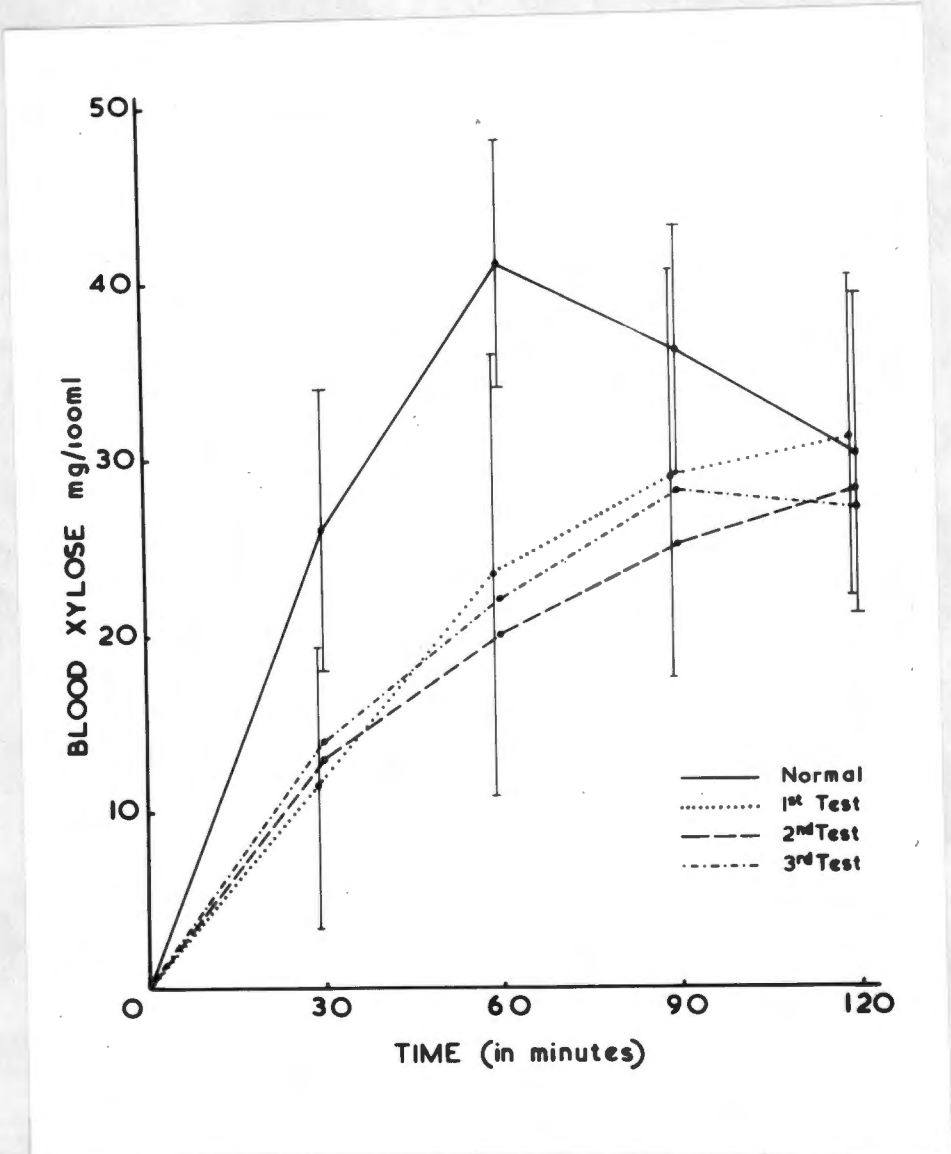


**FIGURE 5.**

**Mean blood xylose levels obtained during the three tests. Vertical bars represent 1 SD above and below the mean. For reasons of clarity only the standard deviation of the first test is included. Standard deviations of the other two tests were almost identical. Normal curve and standard deviation taken from the literature.**

Carbohydrate Tolerance Test:

The results of the carbohydrate tolerance tests are summarised in Table XVI.

Table XVI.

The absorption ratios of sucrose and maltose are all well over 50% of the absorption of glucose/galactose. In over 70% of cases tested the absorption of these particular disaccharides was better than the absorption of glucose/galactose (i.e. over 100%).

Lactose absorption ranged in the whole series from an absorption ratio of 0 to 127% of the monosaccharides. In the patients are divided into the two groups previously described on the basis of stool weights and other evidence of fermentative diarrhoea, the following is found. Of the 7 "absorbers" six had lactose tolerance tests performed and the mean absorption ratio of 72% for this group is well above the arbitary dividing live of 50%.[107] Only two had absorption ratios under 50% (39% and 49%). Of the 13 "non-absorbers", 12 had lactose tolerance tests and all had absorption ratios of less than 50% with a mean value of 19%. The difference between the lactose absorption ratio of the absorbers and non-absorbers is statistically significant. ($p < 0.001$) (Table XVI, Fig.6)

The results of the follow-up studies of absorption ratios of lactose are shown in Table XVII.

Table XVII.

In most there is a tendency to a gradual improvement of absorption but only one (case 6) showed a return to near 50% absorption ratio. In one child (case 10) follow-up was continued for a year after the initial admission and the absorption ratio of lactose was then only 24%. The lactose tolerance test was always followed by a brief episode of diarrhoeic stools.

Nitrogen Balance:

In the 20 children studied the percentage apparent absorption of nitrogen was 79% on the initial milk feeds. When the diarrhoea was controlled by giving the

## TABLE XVI

## CARBOHYDRATE TOLERANCE TESTS.

| Case No. | Absorption Ratio % | | |
|---|---|---|---|
| | Sucrose | Maltose | Lactose |
| 2 | | | 60 + |
| 3 | | | 127 + |
| 4 | | | 39 + |
| 5 | | | 14 |
| 6 | 107 | | 5 |
| 7 | 146 | | 30 |
| 8 | 206 | | 46 |
| 9 | 80 | | 8 |
| 10 | 70 | 89 | 4 |
| 11 | 197 | 71 | 6 |
| 13 | 126 | 124 | 97 + |
| 14 | 269 | 208 | 49 + |
| 15 | 140 | 143 | 58 + |
| 16 | 115 | 102 | 6 |
| 17 | 64 | 63 | 0 |
| 18 | 158 | 109 | 42 |
| 19 | 164 | 147 | 40 |
| 20 | 164 | 179 | 25 |
| Mean | 143 | 124 | 36 |
| Means | 6 Absorbers + | | 72 |
| | 13 Non-absorbers | | 19 |

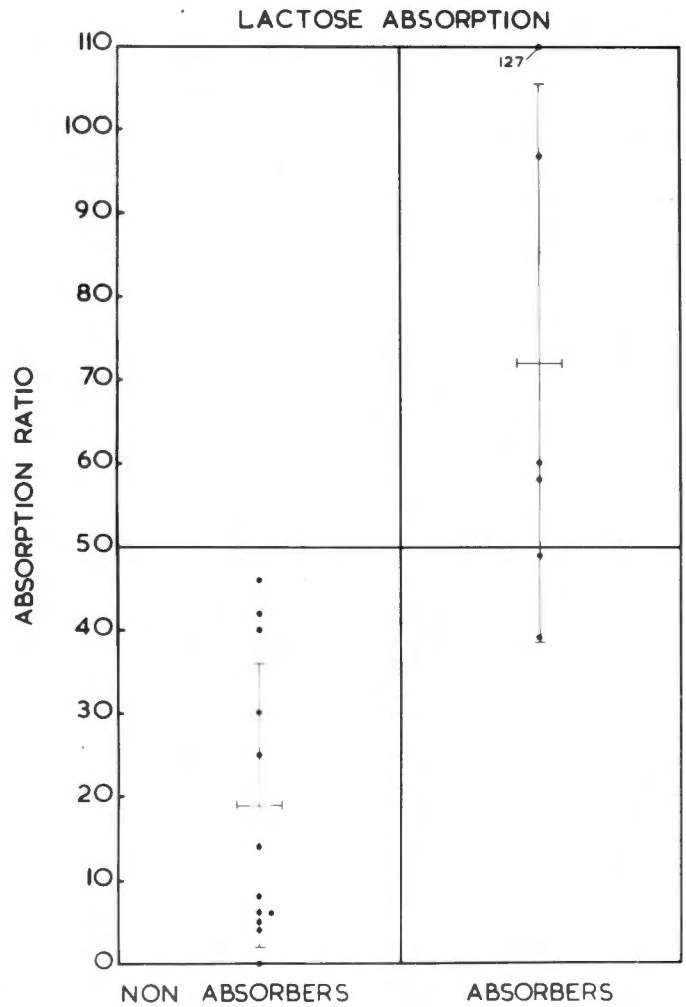


**FIGURE 6.**

**Graphic comparison of lactose absorption ratios in non-absorbers and absorbers. Horizontal bars represent the means and the vertical bars 1 SD above and below the means.**

## TABLE XVII.

## LACTOSE ABSORPTION RATIO - FOLLOW UP.

| Case No. | Months after Admission | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Admission | 1 | 2 | 4 | 5 | 6 | 9 | 12 |
| 5 | 14 | 5 | | | | | | |
| 6 | 5 | 42 | | | | | | |
| 7 | 30 | 38 | | | | | | |
| 10 | 4 | | | 16 | 13 | | 27 | 24 |
| 16 | 6 | | 17 | | | | | |
| 20 | 25 | | 15 | | | 33 | | |

disaccharide-free diet nitrogen absorption was significantly improved to 90% of intake. On being put back on to milk there was a significant deterioration of nitrogen absorption to 83% of intake. (Table XVIII)

Table XVIII.

If the series is divided into "absorbers" and "non-absorbers" as previously described the following results were obtained.

In the 7 "absorbers" there was a significant improvement in apparent nitrogen absorption on changing from milk to a disaccharide-free diet. It rose from 84% to 92% of intake. However, when the diet was again changed to milk there was no deterioration of nitrogen absorption it being 88% of intake.

In the 13 "non-absorbers" apparent nitrogen absorption improved from 76% to 88% and again deteriorated to 80% of intake when put back on to milk and the diarrhoea recurred. The improvement and deterioration in this group was statistically significant.

Comparing nitrogen absorption in the two groups there was no significant difference between "absorbers" and "non-absorbers" while on the initial milk feed or on the disaccharide-free formula but the "non-absorbers" had a significantly lower apparent nitrogen absorption when the diarrhoea recurred with the introduction of milk.

<u>Fat Balance:</u>

Table XIX.

In the whole group the general trend of fat absorption was of gradual improvement during protein refeeding. Fat absorption was initially 73% on milk, 80% on the disaccharide-free formula and 82% on the second period of milk feedings. Although there is a gradual improvement in the percentage fat absorption the differences are not statistically significant.

On dividing the series in "absorbers" and "non-absorbers" the same general trend is noted in both groups. Fat absorption tends to improve during refeeding and

## TABLE XVIII.

## MEAN % APPARENT NITROGEN ABSORPTION.

| | No of Cases | Milk Diet | Dissoch.-free Diet | Milk Diet |
|---|---|---|---|---|
| Absorbers | 7 | 84 ± 5 | 92 ± 5 | 88 ± 4 |
| Non-Absorbers | 13 | 76 ± 15 | 88 ± 4 | 80 ± 5 |
| Total | 20 | 79 ± 13 | 90 ± 5 | 83 ± 6 |

Significance : Student (t test)

| | | | |
|---|---|---|---|
| Total | 79/90 | - | $p < 0.001$ |
| | 90/83 | - | $p < 0.001$ |
| Absorbers | 84/92 | - | $p < 0.02$ |
| | 92/88 | - | Not significant |
| Non-Absorbers | 76/88 | - | $p < 0.02$ |
| | 88/80 | - | $p < 0.001$ |
| Absorbers/Non-Absorbers | 84/76 | - | Not significant |
| | 92/88 | - | Not significant |
| | 88/80 | - | $p < 0.01$ |

## TABLE XIX.

## MEAN % FAT ABSORPTION.

| | No of Cases | Milk Diet | Dimacch.-free Diet | Milk Diet |
|---|---|---|---|---|
| Absorbers | 7 | 82 ± 6 | 88 ± 7 | 86 ± 5 |
| Non-Absorbers | 13 | 68 ± 27 | 76 ± 19 | 80 ± 9 |
| Total | 20 | 73 ± 22 | 80 ± 17 | 82 ± 8 |

Significance : Student (t test)

| | | | |
|---|---|---|---|
| Total | 73/80 | - | not significant |
| | 80/82 | - | not significant |
| Absorbers | 82/88 | - | not significant |
| | 88/86 | - | not significant |
| Non-Absorbers | 68/76 | - | not significant |
| | 76/80 | - | not significant |
| Absorbers/Non-Absorbers | 82/68 | - | not significant |
| | 88/76 | - | not significant |
| | 86/80 | - | not significant |

no significant deterioration of absorption was noted while having diarrhoea on a milk diet.

The fat absorption in the "non-absorbers" was consistently lower than in the "absorbers" during all three diet periods but the differences are not statistically significant.

Mucosal Biopsy and Enzyme Assay:

Of the 11 children in whom small intestine mucosal biopsy and disaccharide enzyme activity assay was performed, three were absorbers and eight were non-absorbers. Disaccharidase units (micromoles of substrate split per minute at 37°C) may be expressed per gram wet weight of intestinal mucosa or per gram mucosal protein content. The results differed with the two methods of expression. When expressed per gram wet weight of intestinal mucosa the following was found. Two of the absorbers (cases 13 and 14) had lactase, sucrase and maltase activity within the normal range obtained by Dahlqvist et al[161] and Sheehy et al.[109] The other (case 15) had low sucrase and maltase activity and borderline lactase activity. All the non-absorbers had low lactase and sucrase activity and in only 2 of the 8 was maltase activity within normal range. (Table XX)

Table XX.

The protein content in this series was found to average 101 with a range from 71 to 217 mg. protein/gram wet weight of mucosa. However, 6 of the 9 cases in which the protein content was estimated had values below 90 mg. and averaged 78 mg. protein/gram wet weight mucosa. In 2 cases there was insufficient homogenate for protein estimation and the average value of 101 mg. has been applied in calculating the disaccharidase activity.

When the disaccharidase units were expressed per gram of mucosal protein the following results were obtained. The 3 absorbers all had normal lactase, sucrase and maltase activity when compared to results in the literature[69,109] and those found

TABLE XX.

DISACCHARIDASE ACTIVITY.

| Case No. | Units per Gram wet weight | | |
|---|---|---|---|
| | Lactase | Sucrase | Maltase |
| 10 | 0 | 0.6 | 5.1 |
| 11 | 0 | 0.7 | 2.7 |
| 12 | 0.06 | 0.6 | 2.4 |
| 13 * | 1.85 | 3.8 | 16.5 |
| 14 * | 1.82 | 9.5 | 62.0 |
| 15 * | 0.2 | 1.5 | 4.9 |
| 16 | 0.05 | 0.25 | 1.82 |
| 17 | 0 | 0.6 | 0.96 |
| 18 | 0.1 | 1.6 | 5.9 |
| 19 | 0.18 | 1.06 | 4.6 |
| 20 | 0 | 0.55 | 3.2 |
| "Normals" | | | |
| Dahlqvist[161] | 0.2 - 19 | 6 - 17 | 13 - 54 |
| Sheehy et al[109] | 0.9 - 28.1 | 2.8 - 58.2 | 4 - 149 |

* Absorbers

in our own laboratory.[184] Of the 8 non-absorbers all had low or absent lactase activity. Sucrase activity was in the normal range in 3, slightly below normal in 2 and low in 3. Maltase activity was within the normal range in all but one case. (Table XXI)

Table XXI.

Three of the children followed (cases 10, 16 and 17) had repeat disaccharidase enzyme assays performed and return of lactase activity to normal was not noted. In one case (case 10) followed on a good protein diet for a year lactase activity was still absent at that time.

Clinical Features of Children in Series II.

These are summarised in Table XXII.

Table XXII.

The mean age was 2 years 3 months with a range from 1 year 3 months to 4 years 1 month. The average weight was 7.9 kg. with a mean percent of expected weight of 63%. Mean serum protein concentration on admission was 4.64 gm. per 100 ml. with a serum albumin concentration of 2.02 gm% (range 0.91 - 3.66 gm%). Mean haemoglobin concentration was 10 gm% with a range from 7.1 to 11.9 gm%.

The 13 non-absorbers had the following features. Mean age was 2 years 6 months with a range from 1 year 3 months to 4 years 1 month. The average weight was 8.0 kg. with a mean percentage of expected weight of 61%. On admission mean serum protein concentration was 4.44 gm% (albumin 1.97 gm%). Mean haemoglobin concentration was 10.6 gm%.

The 7 absorbers had a mean age of 1 year 9 months with a range from 1 year 3 months to 2 years 6 months. The average weight was 7.9 kg. with a mean percentage of expected weight of 67%. On admission mean serum protein concentration was 5.0 gm% (albumin 2.1 gm%). Mean haemoglobin concentration was 9.22 gm%. (Table XXIII)

Table XXIII.

## TABLE XXI.

### DISACCHARIDASE ACTIVITY.

| Case No. | Protein Content mg/g Mucosa | Units per Gram Protein | | |
|---|---|---|---|---|
| | | Lactase | Sucrase | Maltase |
| 10 | 77 | 0 | 24.0 | 183.0 |
| 11 | 75 | 0 | 27.3 | 98.6 |
| 12 | 82 | 1.96 | 19.6 | 78.9 |
| 13 ¤ | 71 | 72.2 | 149.5 | 641.2 |
| 14 ¤ | (101) | 50.1 | 262.0 | 1,711 |
| 15 ¤ | 75 | 7.4 | 55.8 | 182.3 |
| 16 | 98 | 1.5 | 7.1 | 51.2 |
| 17 | 89 | 0 | 18.7 | 30.2 |
| 18 | (101) | 2.8 | 44.6 | 162.8 |
| 19 | 217 | 2.3 | 13.4 | 58.2 |
| 20 | 130 | 0 | 11.9 | 70.2 |
| "Normals" | | | | |
| Shoshy et al[109] | | 4 - 149 | 21 - 247 | 52 - 816 |
| Auricchio et al[69] | | 6 - 54 | 24 - 152 | 70 - 456 |
| Barbezat et al[184] | 70 - 160 | 4 - 73 | 14 - 247 | 68 - 1612 |

¤ Absorbers

## TABLE XXII.

## CLINICAL FEATURES.

## SERIES II.

| Case No. | Age yr/mth | Wt. (Kg.) | % Exp. Wt. | Serum Proteins | | Haematology | |
|---|---|---|---|---|---|---|---|
| | | | | Total | Albumin | Hb. | P.C.V. |
| 1 | 2/0 | 7.82 | 63 | 6.04 | 2.88 | 10.9 | 32.5 |
| 2 | 2/0 | 7.10 | 57 | 3.50 | 1.18 | 8.6 | 34.5 |
| 3 | 1/3 | 8.74 | 82 | 5.94 | 3.66 | 10.6 | 35.6 |
| 4 | 1/9 | 7.76 | 65 | 6.46 | 2.26 | 10.1 | 32.5 |
| 5 | 3/6 | 7.69 | 51 | 6.58 | 3.47 | 11.9 | 36.6 |
| 6 | 1/11 | 4.70 | 38 | 4.11 | 1.43 | - | 25.0 |
| 7 | 2/1 | 6.25 | 50 | 6.02 | 3.48 | 11.3 | 35.3 |
| 8 | 1/11 | 5.85 | 48 | 3.34 | 0.91 | 9.5 | 26.5 |
| 9 | 2/3 | 7.15 | 55 | 3.12 | 1.39 | 10.5 | 35.0 |
| 10 | 2/1 | 10.31 | 82 | 6.83 | 3.48 | 11.1 | 35.0 |
| 11 | 2/3 | 9.09 | 70 | 3.77 | 1.31 | 11.9 | 35.0 |
| 12 | 1/11 | 8.11 | 66 | 3.51 | 1.51 | 10.6 | 35.5 |
| 13 | 1/5 | 8.79 | 79 | 4.75 | 2.01 | 9.45 | 32.0 |
| 14 | 2/6 | 6.95 | 52 | 4.62 | 1.05 | 7.1 | 24.0 |
| 15 | 1/7 | 8.51 | 74 | 3.69 | 1.63 | 7.8 | 24.5 |
| 16 | 4/1 | 11.25 | 70 | 4.07 | 1.57 | 10.9 | 33.0 |
| 17 | 2/3 | 7.82 | 61 | 3.64 | 1.85 | 8.3 | 29.0 |
| 18 | 3/8 | 9.52 | 62 | 3.84 | 1.40 | 10.8 | 30.0 |
| 19 | 1/3 | 6.96 | 66 | 5.65 | 2.86 | 9.8 | 30.0 |
| 20 | 2/11 | 9.56 | 68 | 3.28 | 0.98 | 10.3 | 30.5 |
| Mean | 2/3 | 8.00 | 63 | 4.64 | 2.02 | 10.1 | 32.0 |

## TABLE XXIII.

## COMPARISON CLINICAL FEATURES.

## SERIES II.

| Case No. | Age yr/mth. | Wt. (Kg.) | % Exp Wt. | Serum Proteins | | Haematology | |
|---|---|---|---|---|---|---|---|
| | | | | Total | Albumin | Hb. | P.C.V. |
| Absorbers | 1/9 | 7.95 | 67 | 5.00 | 2.10 | 9.2 | 30.8 |
| Non Absorbers | 2/6 | 8.02 | 61 | 4.44 | 1.97 | 10.6 | 32.0 |

Although the non-absorbers are on the average a little older and slightly more malnourished (as judged by per cent expected weight and albumin concentration) than the absorbers these differences are not significant.

Bacteriology:

Of the 7 children with no signs of lactose malabsorption (absorbers) no pathogens were isolated from the stools of 6. One child had a Salmonella organism isolated from the stool. Of the 13 children with evidence of malabsorption of lactose (non-absorbers), one had a Shigella organism isolated from the stool, one had Giardia lamblia cysts in the stool and two had Trichuris trichiuria ova in the stool.

Stool Lactic Acid Content and Stool pH.

The stool lactic acid content and stool pH were compared in daily stool collections. Where the stool lactic acid content was above 1 gm. per 24 hours the stool pH was almost always below 6. There were only 4 exceptions to this. However there were several stools with a stool lactic acid content below 0.5 gm. per 24 hours where the stool pH was below 6. (Fig.7)

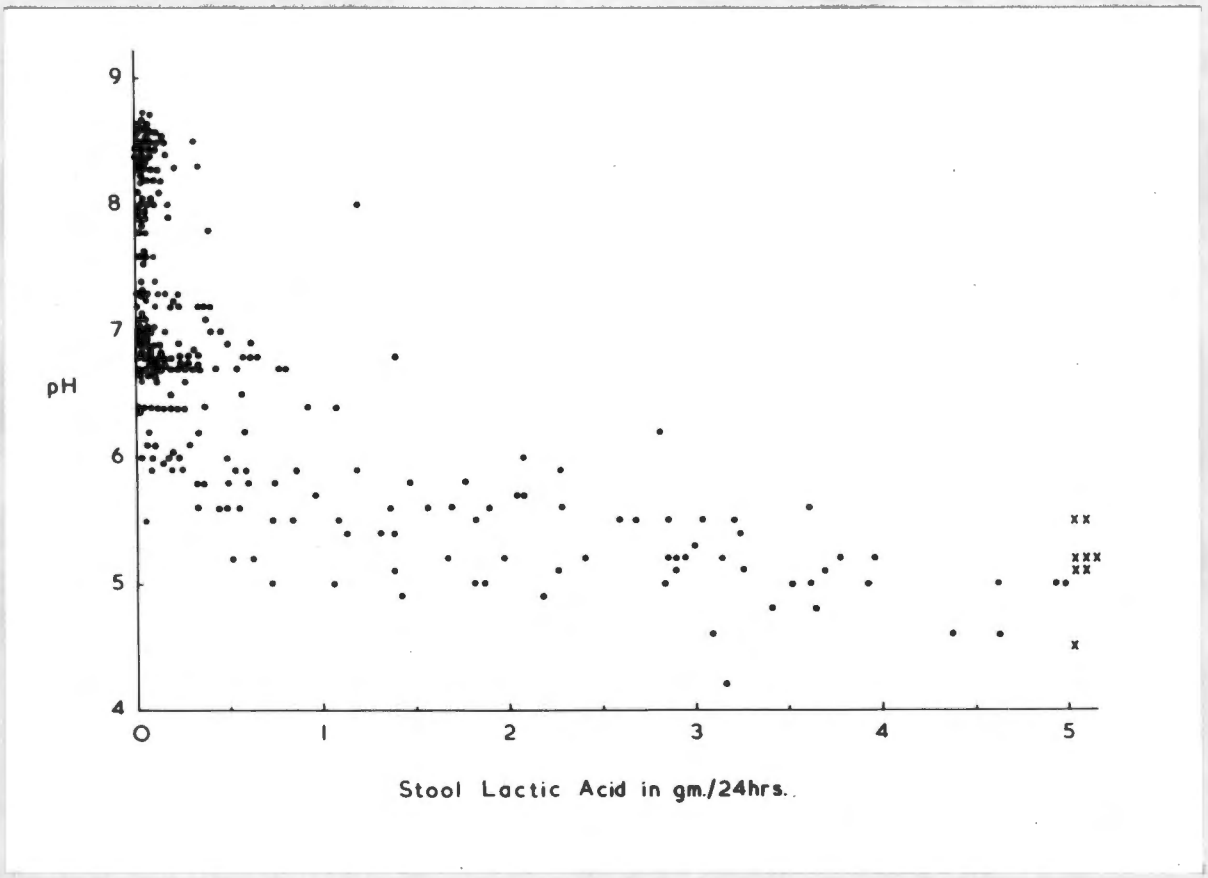


**FIGURE 7.**

**The relationship of stool pH to lactic acid content. Those marked X had stool lactic acid values above 5 gm. lactic acid/24 hours.**

## CHAPTER VI.

## DISCUSSION.


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## CHAPTER VI.

## DISCUSSION.

### The pathogenesis of the diarrhoea in protein-calorie malnutrition.

The effect on diarrhoea on changing from milk to a carbohydrate-free diet was apparent in the first series of children studied. Over half had marked diarrhoea when on milk and the stool weight per 24 hours fell abruptly once they were on a diet free of carbohydrate. On the day of changing the diet the stool weights often remained high. But the following day there was invariably a marked decrease. The abruptness suggests that the elimination of milk from the diet is responsible and it is not an effect of protein refeeding and improved nutrition. On the same antibiotic regimen and carbohydrate containing diets, it has been shown that mean stool weights drop gradually, and only approach near normal values after several weeks.[27] The increase in mean stool weight, when the children initially fed the carbohydrate-free diet were given milk also supports the contention that the diarrhoea is due to the milk, and that the improvement is not due to protein repletion.

From a study of the stool weights alone, it is therefore possible to conclude that a milk diet is at least partially responsible for the diarrhoea in the majority (59%) of the children in Series I. Furthermore the findings of abnormally large amounts of lactic acid and sugars in the stool while on milk, suggests that the diarrhoea is fermentative. The lactic acid content drops to normal levels and sugars disappear from the stool on controlling the diarrhoea by a carbohydrate-free diet. The evidence for a fermentative diarrhoea raises the possibility that the carbohydrate content (lactose) of the milk feeds is responsible for the diarrhoea in the malnourished children studied.

A minority of these children (41%) did not show any change on a carbohydrate-free diet as compared with a milk diet. It is significant that these children did not have severe diarrhoea initially. While on milk, the stool lactic acid content was

not elevated, so there is little evidence that the diarrhoea was fermentative in origin.

In the first series a limited number of investigations were performed to test the hypothesis that malabsorption of lactose was the cause of fermentative diarrhoea. Three children in whom diarrhoea was controlled by the carbohydrate-free diet showed good increments in blood glucose levels following oral glucose/galactose mixtures, but poor increments following lactose administration. One of these children was retested seven months after the initial admission and still had a flat lactose tolerance test, whereas good increments of blood glucose followed glucose/galactose, sucrose and maltose loads. Two children who did not have severe diarrhoea on milk were similarly tested and the blood sugar increment following lactose administration was satisfactory. The results tend to incriminate malabsorption of lactose as the cause of severe diarrhoea.

The second series of children was studied to confirm the above findings and to test the hypothesis that lactose was the cause of the diarrhoea. A feeding schedule of milk, disaccharide-free diet and then a further period on milk, was specifically designed to demonstrate the abrupt changes in stool weight, and to show that improvement in the diarrhoea was not due to recovery with the protein refeeding. The decrease in stool weight and lactic acid content, with the disappearance of sugars from the stool on a disaccharide-free diet in the majority (6/9) of these children suggests that there is a failure of disaccharide absorption. The abruptness of the fall in stool weight on the disaccharide-free formula and the immediate relapse on milk strongly supports this suggestion. The presence of large amounts of lactic acids and sugars in the stool while on milk, again indicates a fermentative diarrhoea. The disaccharide-free formula contained monosaccharide (glucose) and the absence of diarrhoea while on this formula supports the findings in the limited number of tolerance tests done in the first series i.e. monosaccharides are adequately absorbed, but the

disaccharide of milk (lactose) is not.

Again a minority of these children (35%) did not have severe diarrhoea on milk and there was no significant change in stool weights on the disaccharide-free diet. The stool, while the children were fed milk, did not have an elevated lactic acid content, nor were significant amounts of sugar recovered. The mild diarrhoea in some was, therefore, not fermentative and no improvement could be expected on removing disaccharide from the diet.

The more complete range of carbohydrate tolerance tests done in Series II yielded valuable information. No child tested showed any evidence of inability to absorb sucrose or maltose, and administration of these sugars did not induce diarrhoea. The lactose tolerance test and derivation of an "absorption ratio" appear to be a valid way of quantitating malabsorption of this sugar. All the non-absorbers tested (i.e. those with a fermentative diarrhoea) had an absorption ratio below 50% and most were considerably lower ranging from 0-46%. Of the absorbers only one patient who showed no evidence of fermentative diarrhoea on milk had a low absorption ratio (39%). It has been claimed that flat lactose tolerance curves occur in a significant proportion of normal subjects.[53,138,185] From this study it would appear that the finding of a flat lactose tolerance curve and low absorption ratio merits further investigation before dismissing it as a normal variant.

Support of this view comes from the work of McGill and Newcomer.[186] In normal adults they found a significant number of individuals had flat blood glucose curves following a lactose load when the glucose was estimated on venous samples.[185] In the same individuals using capillary samples of blood for the glucose estimation, the flat curves were not found.[186] The blood sugar estimations in all our disaccharide loading tests were done on capillary samples. This probably accounts for their reliability in identifying absorbers and non-absorbers of lactose.

The disaccharidase enzyme assays carried out in the last eleven patients

in the second series also correlate well with the evidence for fermentative diarrhoea on milk and the lactose tolerance tests. This was particularly so when the enzyme activity was expressed as disaccharidase units per gram mucosal protein. All the absorbers had normal disaccharidase activities. The non-absorbers had either absent or low lactase activities. In this group sucrase and maltase activities were low in comparison with the "normals" reported from other centres using an identical assay method.[69,109]

Some authors have expressed disaccharidase units per wet weight mucosa and others per gram protein content of mucosa. It has been stated that the method of expressing the enzyme activities is of little consequence.[109] This does not appear to be valid in the patients with kwashiorkor. Disaccharidase units per wet weight mucosa gave results less consistent with the evidence of fermentative diarrhoea and disaccharide absorption tests, than the other method. The protein content of many of the mucosal biopsies was lower than values reported in the literature.[105] This may be accounted for by the increased total body water of children with kwashiorkor even after they have lost their oedema.[187,188,189]

The low sucrase and maltase activities in children with lactose malabsorption have been confirmed by subsequent studies. In a group of malnourished children with low lactase enzyme activities it was found that sucrase and maltase activities are also low in comparison with our own normals[184] and the normals from other groups using the same assay methods.[69,109] In our patients this depression was not of clinical significance as no evidence was found of inability to deal with sucrose and maltose loads. It probably reflects a generalised depression of all disaccharidase enzyme activities in the affected patients. Sucrase and maltase activities are present in the usual biological overabundance thus no functional effect is noted.

Following our original reports[157,158] of lactose malabsorption as a cause of the diarrhoea of kwashiorkor studies have been done in other centres. In some of these sucrose and even monosaccharide intolerance have been described. Wharton et al[190] working in Uganda found a high incidence of sugar malabsorption and described clinical intolerance of sucrose. Intolerance of monosaccharides (glucose and fructose) was also present in a few children. Lactose intolerance, however, predominated and tended to persist in some children, whereas sucrose and monosaccharide intolerance were less common. In only one child was intolerance of sucrose more than transient. Chandra et al[191] reporting from India, noted sugar intolerance in 50% of malnourished children. Lactose intolerance predominated but intolerance of sucrose, maltose and monosaccharides also occurred. Defects of glucose absorption have been demonstrated by blood glucose studies from Guatemala[192] and by intestinal perfusion studies from Jamaica.[193,194] In the latter,[194] James reports that of 10 children all had diminished glucose absorption, 8 reduced lactose absorption and 6 reduced sucrose absorption.

The finding of clinical intolerance to sucrose and maltose by others[190,191,194] is in accord with our findings of reduced sucrase and maltase enzyme activities in our patients who were clinically lactose intolerant. None of these workers state precisely when their studies were done in relation to admission. Our loading tests were done after 11 days on a high protein intake, and the transient nature of sucrose and maltose intolerance reported, may account for failure to detect them.

The results of the second series confirm the hypothesis suggested by the studies done in the first. Fifty to 60% of the malnourished chidlren studied had diarrhoea because of intolerance to lactose. The evidence of a fermentative diarrhoea, the low disaccharide absorption ratio and the diminished disaccharidase enzyme activity showed a high degree of correlation in the individual child intolerant of lactose.

The duration of the lactose intolerance.

The follow up studies of the lactose absorption were perforce limited in time and numbers. Most of the patients had to be discharged home after recovery and they then returned to the same poor diet that originally caused their admission to hospital. In only a few was it possible to arrange admission to a convalescent home where continuing good protein intake was assured. For humanitarian reasons these few tended to be those who were severely malnourished and had recurrent severe diarrhoea when an attempt was made to put them on a diet which could be managed at home. They are therefore, anything but an unselected group. Only one of these children showed a return of lactose absorption ratio to near normal. The three children followed for five months or longer, showed only an insignificant improvement of lactose absorption. In some of these children therefore, the lactose malabsorption is not transient. One child followed for a year still had unequivocal evidence of lactose malabsorption. It seems unlikely that more prolonged protein refeeding would have brought about a return to normal absorption in this patient.

Wharton et al[190] showed that most of their patients recovered their ability to absorb lactose. Unlike sucrose or monosaccharide intolerance which were transitory, the lactose malabsorption tended to persist for weeks. Chandra et al[191] showed that nutritional recovery reversed the abnormalities in all except four patients. In these four, a primary enzymic deficiency was considered likely because of the persistence. James[194] found that all his patients improved and the disaccharidase activities increased on good nutrition.

Further studies of enzyme activity of intestinal biopsies on admission and at varying periods thereafter have been done in this unit.[195] In about half the cases lactase levels returned to normal within days. If they did not they were often still low when assayed after several weeks. Return of lactase activity to normal occurred after several months in the occasional child. What proportion of

children had persistent and permanent lactose intolerance after an episode of malnutrition is not yet known.

The effect of lactose induced diarrhoea on other nutrients.

The carbohydrate intolerance tests suggested that monosaccharide absorption was reasonably well preserved in the children with kwashiorkor studied. Blood sugar increments were satisfactory following glucose/galactose administration. It was thought that xylose absorption studies might clarify this aspect. A small number of xylose tests in Series I had indicated that the diarrhoea might reduce absorption.

The D-xylose test has become a standard technique in the investigation of small bowel absorptive function. It is uncertain whether absorption of this pentose is an active or a passive process. It is therefore, not a direct measure of monosaccharide (hexose) absorption, but is, nevertheless, widely employed as a test of absorption of simple sugars requiring no digestion. Absorption is independent of bile, intestinal or pancreatic secretions.[166]

In the normal subject it has been shown that the D-xylose absorption and excretion is almost complete in 5 hours.[166,167] There should be no marked difference between the D-xylose content of a 24 hour urine collection and that of an accurate 5 hour collection.[167] However it has been shown in idiopathic steatorrhoea that the low xylose excretion is due to a delay as well as diminished absorption.[196] The use of 24 hour collections would tend to discount the effect of delayed absorption and obscure the separation of normal and abnormal absorption.

In the second series the 5 hour excretion of xylose was significantly less than the 24 hour excretion. A greater number of low values occurred in the 5 hour excretion test. This may be caused by delay in absorption, but it may be argued that this finding was due to the difficulty of an accurate collection of a 5 hour specimen of urine. The total volume of urine secreted by a young child in 5 hours being small, failure to collect even a small amount of urine remaining in

an incompletely emptied bladder would cause a significant reduction in the calculation of the amount of D-xylose excreted.

The possibility that the lower values found in the 5 hour tests reflects a delay in absorption, is supported to a certain extent by the blood xylose results. A blood xylose curve derived from values in healthy infants and children shows peak values 60-90 minutes after ingestion.[169] In our series the mean highest values in all three test periods only occurred 120 minutes after ingestion. The mean values for xylose excretion for both 5 hour and 24 hour tests in all three periods were above the normal 5 hour excretions reported by various authors.[166,167,181-183] It would thus appear that the total amount of xylose absorbed is within normal limits, although there is a possibility that there may be some delay in absorption. The finding of relatively normal xylose absorption correlates well with the finding of satisfactory blood sugar increments following monosaccharide loads. The clinical success of the disaccharide-free diet which contained glucose in controlling the diarrhoea is also in keeping with these findings. We have not encountered monosaccharide intolerance in our patients and the xylose tests suggest that the simple absorptive processes in the upper small bowel are intact. Diarrhoea induced by lactose malabsorption does not seem to have any significant effect on the amount of xylose absorbed.

Steatorrhoea has been previously reported in patients with kwashiorkor.[47] Fat absorption improved on refeeding but even after eleven days (the period during which the diets were alternated) a significant degree of steatorrhoea persisted. In the non-absorbers (lactose intolerant) the control and induction of diarrhoea did not alter the percentage of fat absorbed. The non-absorbers had generally poorer fat absorption than the absorbers, and it could be argued that this reflected the degree of damage to the small intestine mucosa. The differences were not statistically significant and a larger series would have to be done to resolve this point.

Nitrogen absorption results are rather difficult to interpret. In the absorbers there is significant improvement in the apparent nitrogen absorption from the first period on milk to the period on disaccharide-free diet, which was maintained when the patients were put back on milk. This has been interpreted as due to protein refeeding and in fact nitrogen absorption in the last two diet periods reaches normal levels (i.e. 89%).[198] In the non-absorbers there is an improvement of nitrogen absorption on the disaccharide-free diet, but a significant deterioration occurs on the return to milk feeds. This suggests that the lactose induced diarrhoea does diminish nitrogen absorption. Nitrogen absorption is significantly lower in the non-absorbers compared with the absorbers during the second period on milk.

A striking finding is that in all children nitrogen absorption is relatively good after the first few days. The lactose induced diarrhoea does produce a malabsorption of nitrogen but not to a degree that would be of much clinical significance. This is in keeping with the findings of other workers that protein absorption is surprisingly little affected in diarrhoeal disease.[197] Some effect on nitrogen absorption is present and it has also been shown that in very severe diarrhoea loss of nitrogen can be large.[198] A fall in nitrogen absorption, of little significance on a high intake, might well be sufficient to put the patient on a low intake, into negative nitrogen balance.

It is unfortunate that it was not possible to study mineral absorption in these patients. Certainly the lactose malabsorption provoked the passage of very watery stools, and the loss of water via the intestinal route was always large and occasionally enormous. Probably sodium, chloride and bicarbonate losses were also increased but no measurements were made.

<u>Stool pH and lactic acid content</u>.

The relation shown between the stool pH and lactic acid content is in agreement with the findings of other workers.[56,60] A grossly raised lactic acid

content was associated with a pH below 6, and the higher the lactic acid the lower the pH. However, pH values of 6 or even 5 were found in a fair number of stools with a normal lactic acid content. A low pH of the stool is therefore only suggestive that the diarrhoea is fermentative and is not diagnostic. A low pH together with demonstration of sugar in the stool by a simple method (i.e. use of Clinitest tablets as described by Anderson's group[160]) would reliably detect most patients with malabsorption of sugars.

The aetiology of the lactose malabsorption associated with malnutrition.

It has been suggested that a temporary disaccharide intolerance may follow gastro-intestinal infection even in previously well nourished individuals.[57,142,143] All the children in Series I from whom recognised intestinal pathogens were isolated had evidence of a fermentative diarrhoea. Half of the 16 children with fermentative diarrhoea had pathogens in their stools. No pathogens were isolated from any of the 11 patients with no obvious evidence of disaccharide intolerance and fermentative diarrhoea. It seems possible from this that gastro-intestinal infection in a gut previously damaged by malnutrition may be a factor in producing an acquired disaccharide intolerance. It would not be surprising if, in the atrophied gut of protein deficiency, the enzyme mechanisms concerned with the disaccharide absorption were more easily disrupted by infection. The bacteriological studies carried out in Series II failed to support this hypothesis. Intestinal pathogens were found in a minority of both absorbers and non-absorbers. The bacteriological and parasitic studies were carried out by a routine hospital laboratory not specifically engaged in this project. Had this been so, a higher incidence of intestinal infestation and infection in the group with lactose malabsorption might have been shown. As it stands, no convincing evidence has been demonstrated to incriminate infection as an aetiological agent. Others have not demonstrated a higher incidence of enteric infection or infestation in

disaccharide intolerant malnourished children than in those with no evidence of sugar intolerance.[190,191]

The severity of malnutrition did not appear to be significant in the causation of lactose malabsorption. Comparison of the general clinical state in both series showed no significant difference between the two groups. By the criteria used, those who did not have lactose malabsorption were as malnourished as those who did.

There is increasing evidence that genetic factors play a role in determining absence or diminution of lactase activity in the small intestine after the neonatal period. Much of this work has been concerned with primary lactose intolerance in the adult. The original studies were done on adult Caucasian Americans,[109,110] and the incidence of primary lactose intolerance without any other evidence of gastro-intestinal disease was less than 20%. Lactose malabsorption seems to be more common in otherwise normal American Negroes,[107,199-204] Greek Cypriots,[139] certain East African tribes,[141,205-207] South African Bantu,[208] Orientals,[209,210] Asians[140,211-214] and Australian aborigines.[215] Among these groups the incidence of primary lactose intolerance varies from 70% to 90%. The hypothesis has been put forward that the persistence of lactose activity into adult life is found in those groups, communities or races who domesticate cattle and use milk as food. A diminution of lactase activity after the weaning period being in fact the "normal".[216] In the dairy farming races or peoples a mutation has occurred which allows for the persistence of lactase activity throughout life. This mutation confers an evolutionary advantage in that such peoples then have readily available and relatively cheap protein source in the form of cows milk.

All our patients were from the Cape Coloured group whose genetic inheritance includes contributions from Asian, South African Bantu and Caucasian (mainly North West European) sources. No studies of incidence of lactose

intolerance in normal children or adults of this group have been done, but it might be expected to be somewhere between the high incidence of South African Bantu and Asians and the low incidence in Caucasians. In East Africa the Baganda (Bantu) have a high incidence (90%) of adult lactose intolerance and the Hima and Tusi people (Hamitic) have a low incidence (9% and 16%) respectively. The Hutu of Rwanda and the Itu of the Ankole district of Uganda who are assumed to be of mixed Hamitic/Bantu origin have intermediate intolerance levels (33% - 38%).[141,205-207] The same may possibly be true of the Cape Coloured peoples.

The incidence of milk intolerance increases with age in the American Negro.[202] A study of 72 Baganda children showed that newborn infants have normal lactase levels, that with age a flattening of the blood sugar curve in the lactose tolerance test occurs and that most develop lactose intolerance between 3 - 4 years of age.[205] This is the only study defining the age at which lactose intolerance occurs. A proportion of Cape Coloured children may become lactose intolerant at a similar age, but this investigation remains to be done.

Malnutrition may be the prime cause of the lactose intolerance we have studied. Although no functional malabsorption of sucrose or maltose was noted, the sucrase and maltase enzyme activities were depressed in the patients with lactose intolerance. Wharton,[190] Chandra[191] and James[193] have all described intolerance of their disaccharides as well as monosaccharides in malnourished children. This suggests that the low or absent lactase levels are part of a general lowering of disaccharidase enzyme activities secondary to acquired intestinal damage. Protein-calorie malnutrition is associated with atrophy and damage to the small intestine. Although work with rats[217,218] show that protein deprivation does not significantly decrease lactase or other intestinal disaccharidase activities this could well be due to species differences. James[193] concluded from his studies that the generalised nature of the malabsorption and the reversibility of the defects

suggests that the lactose intolerance in the children he investigated was related to the nutritional state and not to a genetic predisposition.

The evidence for a generalised secondary disaccharidase deficiency does not exclude the possibility that lactase deficiency may be of genetic origin. This is particularly so as our studies and subsequent investigations show that in a relatively high proportion of children lactose intolerance persists after the nutritional defect has been remedied. It could be argued that lactose intolerance and the resultant diarrhoea may be a factor in the causation of malnutrition. The fermentative diarrhoea and protein depletion would then cause a depression in the other disaccharidase enzyme activities.

About 40% of the children studied did not have lactose malabsorption and were as poorly nourished as those who did. Lactose intolerance clearly cannot be incriminated as the cause of malnutrition in these and it seems more likely that both groups were a result of poor dietary intake. Malnutrition may well cause a predisposition to early loss of lactase activity to manifest earlier than it otherwise would.

From study of our results and the subsequent work by others, it is concluded that malnutrition damages the gut and causes a generalised secondary disaccharidase deficiency. The finding of persistent lactose intolerance in some, requires further work to elucidate its significance. One can only speculate that in the racial group studied there is an hereditary tendency to lactase loss, whose early appearance is provoked by the insult of malnutrition. The failure of lactase, unlike other disaccharidases, to recover on good nutrition would then be explained on a genetic basis. The persistence of lactose intolerance in a proportion of patients with disaccharidase deficiency in association with other disease (i.e. coeliac disease) could be similarly explained.

The implications of disaccharide intolerance and malnutrition.

The high incidence of disaccharide malabsorption in severely malnourished infants is of importance from a public health point of view. In many areas of the world, improvement of nutrition is most easily accomplished with milk or dried milk products. The lactose present in these may actually increase and perpetuate diarrhoea in some children. The production on a commercial scale of disaccharide-free milk is costly and would not appear to be economically feasible. If malnutrition is the cause of the disaccharide malabsorption, then the use of milk or dried milk products in the prevention, rather than cure of malnutrition would be a sound policy. If malnutrition can be prevented then disaccharide intolerance would not occur and milk could safely be used as a continuing source of good quality and reasonably cheap protein.

It is, therefore, of practical significance to resolve the problem of what role a genetic predisposition to early lactase loss plays in the aetiology of disaccharide intolerance in malnutrition. If primary genetic lactose intolerance does play a role in the causation of malnutrition, then milk would not be of value in prophylaxis. If our speculation that lactose intolerance is caused by the malnutrition and that genetic predisposition to early lactase loss is mainly responsible for the persistence of the defect, then the situation is somewhat different. Little work has been done in normal infants and pre-school children in those races where early hereditary loss is common. If the work of Cook et al[205] in the Baganda children is generally applicable then significant lactose intolerance occurs between 3 - 4 years of age. Milk could be used as a protein source to prevent malnutrition in infants and small children. By the time lactose intolerance might be expected to appear these children would at least have had a good nutritional start, and at that age would naturally be less dependent on milk as their main source of protein.

## SUMMARY AND CONCLUSION.

Throughout the world diseases and disorders in which diarrhoea is an outstanding manifestation, account for many millions of deaths of infants and children every year. The association of diarrhoeal disease and a high mortality in undernourished populations has long been noted, and the relationship between malnutrition and diarrhoeal disease an area of controversy. Some have claimed that the nutritional state is of prime importance whereas others have felt that repeated gastro-intestinal infection and infestation is the more significant and largely accounts for the malnutrition. The low standards of personal hygiene associated with poor socio-economic circumstances are incriminated in the repeated infections. On the other hand, protagonists of the nutritional state being the cause, have shown that growth retardation occurs before the onset of diarrhoea. It is generally agreed that in the developed countries where malnutrition has almost disappeared, diarrhoeal disease still occurs but is a self limiting disease with a negligible mortality. Where malnutrition is common, diarrhoeal disease remains a major cause of death of pre-school children.

It would appear that it is not the precipitating cause of diarrhoea that is important in this context. It seems undeniable that gastro-intestinal infection and infestation cause diarrhoea. It is more relevant to determine what factor makes diarrhoeal disease severe and persistent in malnourished children. The severity and chronicity of the diarrhoea account for the high mortality in malnourished as compared with well nourished individuals.

Diarrhoea is described as an almost constant association of the clinical picture in severe protein-calorie malnutrition. The pathological changes found in the digestive systems of such patients suggests that disturbances of digestion and absorption probably occur. At various times since the beginning

of this century paediatricians have blamed the carbohydrate content of the diet as a cause of diarrhoea. Dean, by clinical observation suggested this was so in malnourished children.

Advances in the understanding of carbohydrate digestion and absorption have been rapid over the past few years. This particularly applies to the disaccharides and the disaccharidase enzymes. Descriptions of clinical syndromes related to the congenital absence of one or more of these enzymes documented the changes that may occur, and laid a scientific basis for the investigation of sugars being a cause of diarrhoea. These descriptions, the observation of watery acid stools and the pathological changes found in the digestive tract in protein-calorie malnutrition led to the present investigation.

This demonstrates that lactose malabsorption is a major cause of severe diarrhoea in malnourished children. The finding is of obvious significance in the treatment and rehabilitation of the individual malnourished child. The diarrhoea of lactose intolerance causes a serious loss of water in the stools. It does not have a marked effect on the absorption of simple sugars or fat, but does on nitrogen absorption. While this might not be of great significance where protein intake is high, it may well be important where protein intake is marginal. The diarrhoea would increase protein depletion and be a factor in worsening the nutritional state.

The discovery of lactose intolerance in protein-calorie malnutrition acquires added significance when considered in the context of programmes to eliminate nutritional disease. It could be argued that the high incidence of lactose malabsorption renders milk an unsuitable source of protein for the cure of malnutrition and will induce diarrhoea in many malnourished children. It is not easy to find an adequate substitute and milk remains the most available and cheap protein source. Nitrogen absorption is usually good and it is possible to achieve protein repletion despite the induction of diarrhoea. As nutritional recovery occurs many

will regain lactose tolerance and the diarrhoea will cease.

The low or absent lactase activities appears to be part of a generalised disaccharidase deficiency secondary to protein-calorie malnutrition. Depression of other disaccharidase enzyme activities occurs, but only lactose malabsorption is functionally apparent. Lactase is normally present in smallest amounts and it requires a considerably greater depression of other disaccharidase activities before functional effects of malabsorption become apparent. The use of milk in prevention rather than cure of malnutrition would still appear to be sound policy. If malnutrition can be prevented then sugar intolerance will not occur.

In this regard it becomes crucial to define more precisely what role an hereditary predisposition to early lactase loss plays in the lactose intolerance of severe protein-calorie malnutrition. Studies are needed to define when it normally occurs in various racial groups and whether it plays a part in causing malnutrition or whether malnutrition causes it to appear early. It is only possible to speculate that the latter seems the more likely and that the malnutrition is due to inadequate dietary intake. The persistence of lactose malabsorption in some children after nutritional recovery may be on a genetic basis.

In the present state of our knowledge, State and International agencies should continue to advocate the use of milk in programmes to eliminate malnutrition. It is usually the cheapest and often the only available source of protein. In the pre-school child it will effectively prevent protein-calorie malnutrition although its role in an established case is less certain. Many can be cured even if diarrhoea is induced, but in some the diarrhoea will be unacceptably severe. For these cases, efforts should be intensified to produce an economical lactose free milk or an alternative protein source.

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